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#### COVER: Cychrus tuberculatus (Coleoptera: Carabidae)

Photographed on Triquet Island off the central coast of British Columbia. This rare woodland beetle slowly meandered through the mossy understory of a cedar forest, unaware of the 14,000-year-old Heiltsuk village hidden in the earth beneath its feet.

#### Photograph details:

Photograph by Crystal Ernst, Hakai Institute/Simon Fraser University. Canon PowerShot SX10 IS, with Raynox DCR-250 clip-on macro lens, f/4.5, exposure 1/40, ISO 200, natural light.

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## Journal of the LIBRARIES Entomological Society of British Columbia

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## Grape leaf rust mite, *Calepitrimerus vitis* (Acari: Eriophyidae), a new pest of grapes in British Columbia

L. B. M. JENSEN<sup>1,2</sup>, D. T. LOWERY<sup>1,2,3</sup>, and N. C. DELURY<sup>1</sup>

#### **ABSTRACT**

The grape leaf rust mite, *Calepitrimerus vitis* (Nalepa), was first discovered in the interior of British Columbia in 2009 on grape leaves from a commercial vineyard north of Osoyoos. Bronzing of grape leaves confirmed to be caused by *C. vitis* in summer 2009 was followed by severely stunted shoots and distorted leaves in several vineyards in spring 2010. Numbers and lengths of shoots and fruit clusters were reduced significantly on vines infested with *C. vitis*. Earlier studies have shown that outbreaks of *C. vitis* result from pesticide sprays targeted to other pests that damage predator mite populations. Sprays of sulphur-based fungicides early in the season are the recommended method of control.

#### **INTRODUCTION**

Grape leaf rust mite, *Calepitrimerus vitis* (Nalepa) (Eriophyidae), is a host-specific pest of grapevines, *Vitis vinifera* L., (Anonymous 1968; Bernard *et al.* 2005; Walton *et al.* 2007) found in most grape-growing regions of the world, including Washington State since 2002 and Oregon since 2004 (Prischmann and James 2005; Walton *et al.* 2007). *Calepitrimerus vitis* has not previously been reported on grapevines in British Columbia (B.C.) and was not found during an extensive survey of vineyard pests conducted in the Okanagan and Similkameen valleys in 1972 (Madsen and Morgan 1975).

Calepitrimerus vitis has been considered an economic pest of grapes only during the past four decades, possibly the result of reduced use of sulphur-based fungicides that provide effective control (Anonymous 1968; Barnes 1970; James 2007) and from increased applications of pesticides that are harmful to predators that normally keep its numbers in check (Winkler et al. 1972; Bernard et al. 2005; Schreiner et al. 2014). Bronzing of grape leaves in late summer can appear significant but is not thought to affect the current year's growth or quality of the fruit at fall harvest (Anonymous 2005; Reinert 2006; James 2007). However, leaf bronzing is a good indicator of the potential for large overwintering rust mite populations to emerge the following spring and continue feeding, resulting in damage to the developing buds, shoots and leaves (Bernard et al. 2005; Prischmann and James 2005; James 2007). Significant economic injury can occur to grapes if these mites are not properly managed. Feeding of overwintered C. vitis in spring on developing buds and shoots results in what has been termed short shoot syndrome or reduced spring growth (Bernard et al. 2005; Walton et al. 2007; Schreiner et al. 2014), which is typified by severely stunted growth, shortened internodes, scarring of shoots, curled and distorted basal leaves, and reduced fruit set. Severe infestations can result in abortion of affected bunches and complete crop loss (Walton et al. 2007). The relationship of impaired and damaged spring growth of grapevines to C. vitis feeding is not always clear. Several other factors may also be responsible for restricted spring growth, such as heavy thrips (Thysanoptera) feeding, winter freeze and herbicide damage (Schreiner et al. 2014).

This paper documents the first confirmed discovery of *C. vitis* in the Okanagan Valley of B.C. and the results of subsequent surveys to assess *C. vitis* abundance and its impact

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on vine growth. Grape growing and wine production are important industries in the Okanagan Valley, with 8,060 acres currently planted in wine grapes, accounting for 84% of B.C.'s vineyard acreage (British Columbia Wine Institute 2015). In order to provide advice to growers on the best management practices for this emerging pest, we also present information on recommended methods of control developed elsewhere that mostly rely on early season applications of sulphur.

#### **MATERIALS AND METHODS**

Identification of Calepitrimerus vitis. Inspection on June 24, 2009, of grapevines at a commercial vineyard north of Osoyoos (49° 05' 23" N, 119° 30' 39" W) that had heavily bronzed leaves revealed the presence of eriophyid mites. Samples of these mites were preserved in 70% ethanol and sent for species verification to Dr. F. Beaulieu, Canadian National Collection of Insects, Arachnids and Nematodes, Agriculture and Agri-Food Canada, Ottawa, and to Dr. J. Amrine, West Virginia University.

**2009** Summer Survey. A survey for *C. vitis* was conducted during June 24 to September 8, 2009, in twelve vineyard cultivar blocks in eight vineyards located throughout the southern half of the Okanagan Valley from Summerland and Naramata in the north to Osoyoos in the south (Figure 1), including three blocks (Osoyoos: Shiraz, Merlot and Cabernet Sauvignon) adjacent to the vineyard block originally found to be infested on June 24. Two of the original Osoyoos blocks and a heavily infested site in Naramata were sampled 2–3 times over the course of the field season. Leaf samples consisted of 10 randomly selected leaves from each cultivar block. As per the protocol of Walton *et al.* (2007), mites from a sample were transferred to a glass plate by means of a mite brushing machine (J. G. H. Edwards, Okanagan Falls, B.C.); the glass plate was previously covered with a thin film of soap to immobilize the mites. The glass plate was then placed on a grid and examined using a dissecting microscope for counting all mite species and developmental stages.

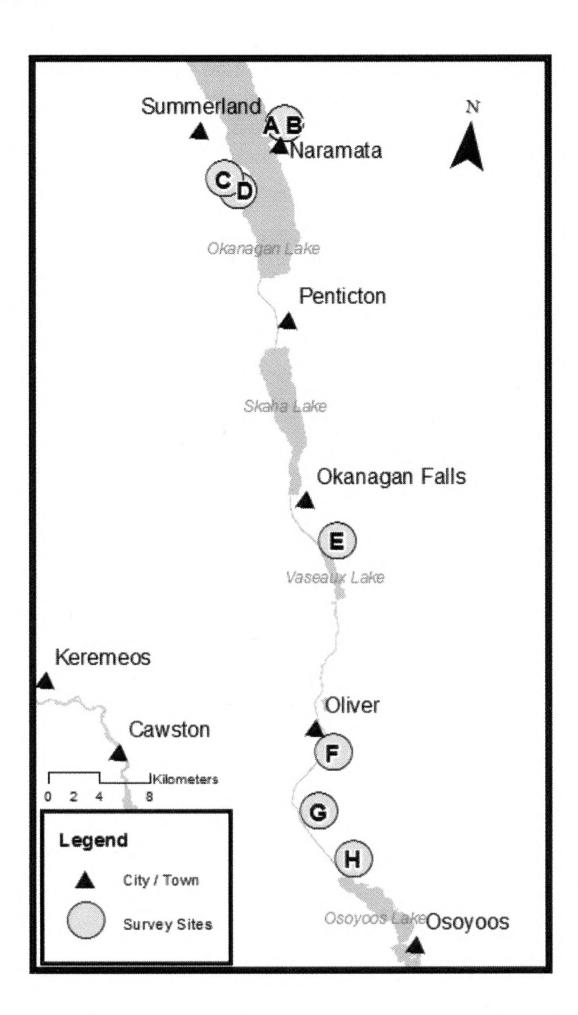
2010 Spring Bud Dissections. In early spring 2010, buds from four vineyards found to be infested with *C. vitis* in 2009 were dissected, and all mite species under individual outer bud scales were counted. Vineyard managers of three of the four sampled vineyards applied sulphur (Kumulus; 80% sulphur; 2.86–11.2 kg/ha; BASF) during the woolly bud stage of development as per the recommended method of control (Anonymous 1968; Bernard *et al.* 2005). Ten randomly selected canes from each sampled cultivar block were pruned above the third bud and temporarily placed in cold storage for no more than one week until the assessments. Mite counts were conducted on the most basal bud from each pruned cane. This procedure was conducted once in March before the sulphur sprays and again in April approximately two weeks after the sulphur sprays.

**Damage Assessment.** The effect of early season feeding by *C. vitis* on developing shoots and fruit clusters was determined in a block of Chardonnay grapes at Okanagan Falls that had one section of eight vine rows heavily infested with *C. vitis* and showing symptoms of spring feeding damage (i.e., stunted and scarred shoots, small distorted leaves, brown and shrunken fruit clusters, etc.). The two adjacent damaged and undamaged sections of the block had been managed in exactly the same manner, except that the vineyard manager had sprayed the heavily infested rows the previous year with an undisclosed insecticide to control leafhoppers. No information about the spray application was provided. The use of a recapture sprayer for the insecticide application resulted in a clear differentiation between rows with and without *C. vitis* feeding damage.

Shoot lengths and counts of *C. vitis*, phytophagous thrips, and predatory mites on stems and the basal leaf of 20 randomly selected shoots per row were assessed on May 18, 2010, from four of the damaged and four of the undamaged rows. Predatory mites and phytophagous thrips were not identified to species. Data were collected from the third to sixth row away from the dividing line between the adjoining damaged and undamaged sections. Pre-harvest data were collected from half (one arm) of each of 20

randomly selected vines per row from those same four rows, with numbers of shoots and clusters counted and cluster lengths measured on September 15, 2010.

**Statistical analysis.** Shoot lengths, mite and thrips counts, and pre-harvest data from damaged and undamaged vine rows were analyzed using one-way ANOVA. All mite and thrips count data were transformed ( $\sqrt{(X + 0.5)}$ ) before analysis (Zar 2010). Statistical tests were performed using JMP Version 10 (SAS Institute Inc. 2013), with all statistical error rates at  $\alpha = 0.05$ .



**Figure 1.** Locations of vineyards surveyed for *Calepitrimerus vitis* in the south Okanagan Valley, B.C. Counts of *C. vitis*, predatory mites, and tetranychid mites (numbers/leaf) for corresponding cultivars at each survey site are located in Table 1.

Mean numbers of Calepitrimerus vitis [Cv], predatory mites [PM] and tetranychid mites [TM] per leaf from 12 cultivar blocks in eight vineyards in the Okanagan Valley, BC, sampled<sup>a</sup> between June 24 to September 8, 2009.

ot, sample	oc, sampled cenween June 24 to September 6, 2009.	epicinosi o, 2003.						
Map Label <sup>b</sup>	Location	Variety	Sample Date (2009)	Cv/leaf	PM/leaf	PM eggs/leaf	TIM/leaf	TM eggs/leaf
	Naramata	Merlot	20-111	269.2	0		7.7	5.2
A		=	Aug-05	0.866	1.8	7,	11.0	12.4
В	Naramata	Pinot Gris	11-14	0	0	0	12.8	30.2
C	Summerland	Gewürztraminer	Jul-15	0	0	0	0	0
D	Summerland	Gewürztraminer	Sep-08	54.8	0	0	0	0
Ш	OK Falls	Pinot Noir	Jun-30	0	0	0	0.1	0
<u> </u>	Oliver	Chardonnay	Jul-15	0	0	0	0	0
H	Oliver	Merlot	Jul-15	0	0	0	0.2	3.2
Ð	Oliver	Semillon	Jul-15	0	0	0	4.0	0.4
Ŋ	Oliver	Shiraz	Jul-15	0	0	0	0	0
Н	Osoyoos	Cabernet Sauvignon	Jun-24	2.3	0	0	0.1	0.1
Н	Osoyoos	Merlot	Jun-24	0.5	0	0	0	0
工	ε	ε	Sep-02	19.0	0	0	0.2	6.4
H	Osoyoos	Shiraz	Jun-24	139.6	0	0	0	2.0
H	ε	Ξ	Aug-05	191.0	0.4	0	11.0	21.2
I	ε	ε	Sep-02	55.2	0.	0	4.0	9.9
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<sup>a</sup>Mites removed from ten randomly selected leaves per block using a mite brushing machine. <sup>b</sup>Letters correspond to vineyard locations on Figure 1.

#### **RESULTS AND DISCUSSION**

Identification of Grape Leaf Rust Mite. The eriophyid mites found to be the cause of bronzing or russeting of grape leaves in a commercial vineyard north of Osoyoos in the south Okanagan Valley in the summer of 2009 were confirmed by two independent experts to be *C. vitis* (see methods). This is the first record for B.C. Adults of this eriophyid mite species, a relative of the grape erineum mite, *Colomerus vitis* (Pagenstecher) (Eriophyidae), are approximately 0.15 mm long, light amber in colour, broader at the front end, and somewhat wormlike in appearance (Lowery 2015; Figures 2, 3). Feeding by *C. vitis* during the summer causes bronzing or stippling that can appear similar to spider mite (Tetranychidae) injury. Damage to leaves by *C. vitis* feeding is mostly restricted to the upper leaf surface rather than the lower surface and, unlike tetranychid mites, *C. vitis* does not produce webbing.

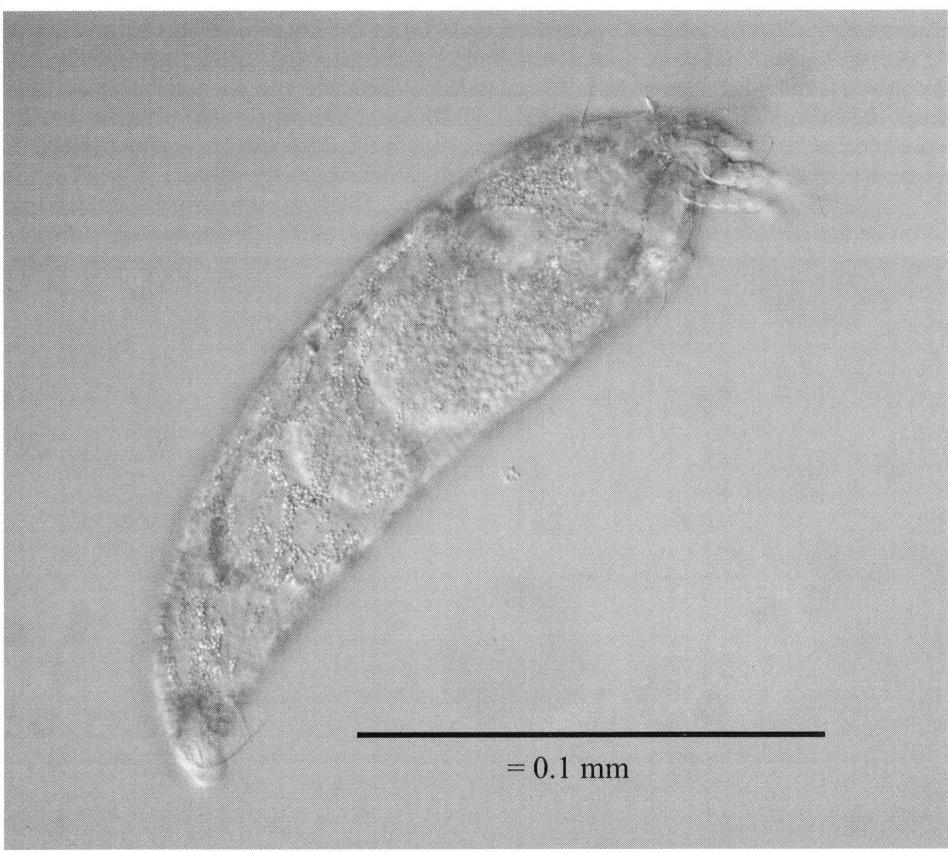


Figure 2. Adult grape leaf rust mite, Calepitrimerus vitis (Nalepa).

**2009** Summer Survey. Of the eight vineyards sampled, *C. vitis* was found in several geographically separated locations (Table 1). Subsequent sampling has found it to be widespread in the valley with numbers as high as 998 mites per leaf. Numbers exceeding 3,000 per leaf have been recorded in Europe and Oregon on severely infested grapevines (Schreiner *et al.* 2014). The presence of *C. vitis* in multiple locations suggests that it has been present in south central B.C. for several years, but widespread movement by wind and with human activities (Duffner *et al.* 2001) could have resulted in rapid dispersal. Monitoring of *C. vitis* on three occasions in one of the surveyed blocks showed that

numbers increased by early August and then declined in September (Table 1). Migration of *C. vitis* from leaves to their overwintering sites under outer bud scales and bark after the end of August agrees with reports by Walton *et al.* (2007) and Schreiner *et al.* (2014).

Grape varieties vary in their susceptibility to *C. vitis* feeding (Anonymous 2005; Bernard *et al.* 2005; Schreiner *et al.* 2014). Combined with cool spring temperatures, cultivars that develop slowly are exposed to the mites for a longer period and so damage may be more severe. Cabernet Sauvignon, a later developing cultivar, is reportedly more susceptible to *C. vitis* than earlier cultivars such as Chardonnay (Anonymous 2005), but our survey did not find large numbers of *C. vitis* on a Cabernet Sauvignon block adjacent to an infested Shiraz block (Table 1). Of the three cultivars sampled in the original vineyard, Shiraz leaves had the largest numbers of *C. vitis*. A Merlot block in Naramata also had high numbers of *C. vitis*. A larger study would be required to determine differences in susceptibility to *C. vitis* among cultivars under southern B.C. conditions.

There was no clear association between *C. vitis* numbers and numbers of tetranychid mites. The highest tetranychid counts (12.8/leaf and 30.2 eggs/leaf) were recorded in a vineyard (site B) where *C. vitis* was not found (Table 1). The second highest number of *C. vitis* (191) occurred on August 5 in a block of Shiraz (vineyard H) that initially had few tetranychids (2.0 eggs/leaf), but high tetranychid numbers were recorded from that same site in August and September. Although low numbers of *C. vitis* were most often associated with low numbers of tetranychids, the complexity of mite population dynamics combined with differing spray regimes confounds the relationship.



Figure 3. Overwintered adult female (deuterogyne) grape leaf rust mites, *Calepitrimerus vitis* (Nalepa), feeding under grapevine bud scales in spring.

2010 Spring Bud Dissections. C. vitis were found in large numbers in spring under the outer bud scales (Figure 3) of vines whose leaves had become bronzed the previous summer. Monitoring of eriophyid mites is difficult due to their microscopic size and cryptic nature (Schreiner et al. 2014). As an alternative to bud dissections, mite counts on

double-sided sticky tape applied around the bases of developing shoots has been used to monitor *C. vitis* emergence in spring (Bernard *et al.* 2005), but the method was not found by Walton *et al.* (2007) to provide useful monitoring information. Scouting for signs of leaf bronzing or stippling in summer followed by an assessment of mite numbers provides a good indication of the need for control the following spring (Schreiner *et al.* 2014). As an alternative to a mite-brushing machine, Schreiner *et al.* (2014) developed a 'rinse in bag' system that extracted *C. vitis* from leaves into a small amount of ethanol or isopropanol for counting.

Sprays of sulphur-based materials during the woolly bud stage of grape development have been shown previously to effectively control *C. vitis* and prevent damage to developing shoots (Anonymous 1968; Bernard *et al.* 2005). Our dissections of buds in spring also indicated that sulphur (Kumulus<sup>TM</sup>) applied by growers at the woolly bud stage was effective against *C. vitis*, as none were detected in the sprayed commercial vineyard blocks two weeks post-application (Table 2). For the cultivar block not sprayed with sulphur, *C. vitis* numbers increased ca. 29% over the same two-week time period. While not as effective, a single application of sulphur in mid-season was reported to reduce *C. vitis* populations by approximately 80% (Schreiner *et al.* 2014). Outbreaks of *C. vitis* in Washington State have been attributed to decreased use of sulphur for powdery mildew control (Prischmann and James 2005; Reinert 2006; James 2007). While reliance on sulphur sprays in the past for the control of fungal pathogens may also have provided control of *C. vitis*, high application rates can be detrimental to predacious mite populations (McMurtry *et al.* 1970; James 2007).

Predacious phytoseiid mites are known to provide effective control of eriophyid mites in the absence of insecticide sprays that are detrimental to their survival (James and Whitney 1993; Bernard *et al.* 2005). In the absence of sulphur sprays, conditions are right for *C. vitis* outbreaks following sprays that are harmful to mite predators. It was apparent, for example, that the application of an undisclosed insecticide to part of a cultivar block resulted in elevated numbers of *C. vitis* and significant damage to developing shoots (Figure 4) the following spring. Differences in *C. vitis* numbers between the heavily infested Shiraz block and the adjoining Merlot and Chardonnay blocks (Table 1) possibly reflects differing pesticide applications the preceding summer. Population levels of *C. vitis* would also vary depending on the frequency and timing of sulphur applications in spring.

**Damage Assessment.** C. vitis may have an uneven distribution even within a vineyard cultivar block. Assessment in spring 2010 of shoot growth in a section of a Chardonnay block that had been sprayed with an insecticide the previous summer showed severe stunting of shoots and distorted leaves (Figure 4). Differences in shoot lengths for the rows heavily infested with C. vitis were significantly shorter than those from the adjacent unsprayed rows ( $F_{1,76}$ =63.3543, P<0.0001) (Table 3). Examination of these damaged, or reduced, shoots showed significantly higher populations of C. vitis on the stems ( $F_{1,76}$ =76.5591, P<0.0001) and basal leaf ( $F_{1,76}$ =46.1787, P<0.0001) compared to undamaged shoots from the same vineyard block (Table 3). Numbers of phytophagous thrips were low, less than one per basal leaf or shoot, and were not found to differ significantly between the damaged and undamaged shoots ( $F_{1,76}$ =2.1008, P=0.1513) (Table 3); therefore, thrips are unlikely to have contributed to the damage. Injury was still measureable at harvest in September, with the C. vitis-infested vines having fewer shoots ( $F_{1,78}$ =5.5237, P=0.0213), fewer grape clusters ( $F_{1,78}$ =102.7369, P<0.0001), and shorter cluster lengths ( $F_{1,78}$ =18.3596, P<0.0001) (Table 3).

Table 2

Numbers (mean ± SE) of Calepitrimerus vitis (Cv) and predatory mites (PM) per grapevine bud scale recorded from four commercial vineyards. All vineyards were previously found to be infested with C. vitis in 2009 and were sprayed or not sprayed with Kumulus (80% sulphur) at the woolly bud stage in spring 2010 as part of individual growers' pest management programs. Mites were counted from under the outer bud scales of the third bud; canes were pruned above the second bud prior to the Kumulus spray and two weeks post-spray and temporarily stored at 2° C.

Sulphur spray	Vineyard site (Map Label")	Cultivar	Date of pre- spray counts (2010)	Pre-spray counts	counts	Date of post- spray counts (2010)	Post-spray counts	y counts
			and the second s	Cv/bud	PM/pre	·		PW/bild
Yes	Osoyoos (H)	Shiraz	March 24	15.56 (± 5.15)	0.22 (± 0.15)	April 28		0.89 (± 0.35)
Yes	Naramata (A)	Meriot	March 10	10.90 (± 2.56)	$0.10 \pm 0.10$	April 29	0	0.90 (± 0.52)
Yes	Naramata (A)	Cabernet Franc	March 10	13.50 (± 4.94)	$0.10(\pm 0.10)$	April 29	0	0.60 (± 0.30)
ŝ	Summerland (D)	Gewürztraminer	March 30	27.70 (± 5.86)	0	April 21	35.80 (± 15.36)	0

\*Letters correspond to vineyard locations on Figure 1.

It is worth noting that the damaged shoots had lower numbers of predator mites than the undamaged shoots, but the difference was not found to be statistically significant (F<sub>1,76</sub>=3.6261, *P*=0.061). Previous work has shown that preservation of mite predators is important for the management of *C. vitis* (James and Whitney 1993; Bernard *et al.* 2005; Reinert 2006; James 2007). James *et al.* (2002) report that effective biological control of eriophyid mites in Washington vineyards likely depends on a complex of natural enemies in addition to species of predatory phytoseiid mites. Outbreaks of *C. vitis* in Australia and Washington State have been attributed to several causes, including the use of broadspectrum, persistent insecticides that harm predators. James (2007) suggested that the appearance of *C. vitis* in Washington State might prove advantageous for grapevine biological control programs, as these mites provide an early season food source for predacious mites before *Tetranychid* spider mite species appear later in the season. Additional study is required to determine the mite predator complex in B.C. and to establish their role in the sustainable management of *C. vitis*.



**Figure 4.** Developing grapevine shoot severely damaged by grape leaf rust mite, *Calepitrimerus vitis* (Nalepa), feeding in early spring (left) compared with an undamaged shoot (right) from the same cultivar block. Note the shortened internodes, brown and distorted leaves and flower buds, and scarring of the stem on the damaged shoot.

In conclusion, our research has demonstrated that reduced spring growth of shoots having deformed leaves that had often been attributed to herbicide or winter damage, post-harvest water stress, thrips, and other maladies is in many cases due to feeding damage from *C. vitis*. Presence of this eriophyid mite under bud scales and on developing shoots has been linked to short shoot syndrome of grapevines, an economically important

syndrome of grapes in the Pacific Northwest of the United States (Walton *et al.* 2007; Schreiner *et al.* 2014). We determined that *C. vitis* were distributed widely in the southern interior of B.C. and observed high numbers feeding on buds and tender shoots in spring (Figure 3) that resulted in distortion and stunting of shoots (Figure 4; Table 3). Although growth often improves during the summer, yields will be reduced significantly, as we have shown.

The recommendation to apply sulphur at the woolly bud stage based on bronzing of leaves in late summer is supported by our observations. Evidence for potentially damaging populations of these mites based on bronzing of leaves in late summer is an indication that control measures should be applied the following spring. Although there is no reported damage to grapes from *C. vitis* feeding during the summer, they can be controlled at this time with foliar sprays of miticides (Anonymous 1968; Walton *et al.* 2007; Siquera *et al.* 2016).

With the documented arrival of *C. vitis* to the southern interior of B.C., it is important that growers learn to recognize early signs of severe *C. vitis* infestations (russeting of leaves) that indicate the need for timely and appropriate sulphur sprays during early bud development the following spring. Attempts should also be made to preserve mite predators by avoiding the use of harmful sprays.

#### **ACKNOWLEDGEMENTS**

We extend our gratitude to Dr. F. Beaulieu, AAFC Ottawa, and Dr. J. Amrine, West Virginia University, for identification of *C. vitis*. Thank you to M. Weis of AAFC-Summerland RDC for photographs, P. Bowen and B. Estergaard, AAFC-Summerland RDC for use of the map, M. Watson of Constellation Brands International, and other producers for allowing access to their vineyard sites.

Table 3

heavily infested with C. vitis compared with undamaged rows from the same Okanagan Falls cultivar block was followed by measures in fall<sup>b</sup> of numbers of shoots, grape Calepitrimerus vitis [Cv], phytophagous thrips, and predatory mites [PM] on four rows of Chardonnay grapes Assessment in springa of shoot lengths and counts of clusters and cluster lengths (means  $\pm$  SE).

			Spring Sho	Spring Shoot Assessment			À	Pre-harvest Assessment	ment
Vine Row Shoot Status	shoot length (cm)	Cv/stem	Cv/basal leaf	thrips/stem	thrips/basal leaf	PW/shoot	shoots (number)	grape clusters (number)	cluster length (cm)
Undamaged	9.98 (± 0.61)		0	0.16 (± 0.06)	0.11 (± 0.06)	0.32 (± 0.10)	0.32 (± 0.10) 8.3 (± 0.38)	10.05 (± 0.66)	10.2 (± 0.57)
Cv-damaged	4.90 (± 0.23)	17.6 (± 2.91)	7.6 (±2.91) 9.48 (±2.11)	0.35 (± 0.09)	0.18 (± 0.08)	0.10 (± 0.05)	0.10 (± 0.05) 7.15 (± 0.31) 2.7 (± 0.31)	2.7 (± 0.31)	6.56 (± 0.57)
Significance level <sup>c</sup>	**	*	*	ns		20	*	*	**

<sup>5</sup>Measures from one cordon arm (1/2 vine) from 20 randomly selected vines per row on September 15, 2010. «Counts on stems and basal leaves of 20 randomly selected shoots per row on May 18, 2010.

\*\*Level of significance assessed with a one-way analysis of variance (a=0.05) and indicated as follows: ns, P>0.05; \*, P<0.05; \*\*P<0.01; \*\*\*P<0.001. All mite count

data was transformed before analysis ( $\sqrt{(X+0.5)}$ ).

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# Insect taxa named for the Rev. John H. Keen, early naturalist on the Queen Charlotte Islands and at Metlakatla, British Columbia

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#### **ABSTRACT**

The Reverend John Henry Keen (1851–1950) spent nearly 20 years serving Anglican missions in British Columbia, at Masset on the Queen Charlotte Islands/Haida Gwaii in the 1890s, and on the adjacent mainland at Metlakatla, during the summer of 1890 and for several years in the early 1900s. Despite leading the busy life of a clergyman, Keen assembled extensive collections of natural history specimens, particularly of insects and mammals. He was spurred on by the likelihood that many specimens would represent species new to science, predictions that were later borne out. Keen initially sent specimens to the Natural History Museum in London, but later sent most of them to Dr. James Fletcher, Dominion Entomologist, in Ottawa, who forwarded many specimens to specialists in the United States and France for identification. Keen was among the first collectors of natural history specimens on the north coast of British Columbia and, in recognition of his contributions, eight insect taxa were named after him, based on the type specimens he collected in this region.

**Key words:** British Columbia; Masset; Metlakatla; Queen Charlotte Islands/Haida Gwaii; *keeni*; type specimens

Among the members of the clergy whose early entomological contributions in British Columbia were chronicled by Riegert (1991) in *Entomologists of British Columbia* was the Rev. John Henry Keen (1851–1950). Keen was an Anglican missionary who served at missions on the Queen Charlotte Islands/Haida Gwaii at Massett (hereafter the modern spelling of Masset is followed; 54.0115°N, 132.1472°W) in the 1890s, and on the mainland coast at Metlakatla (54.3373°N, 130,4447°W) for several years in the early 1900s. Keen arrived in British Columbia in the early summer of 1890 and worked for several weeks on the mainland (Sealy 2016a, b), where he wasted no time in assembling the first collection of beetles from this region (Keen 1891). Keen finally arrived in Masset in mid-September 1890 and began his clerical duties, but also continued what would become a productive period in the history of natural history in this region (Sealy 2015, 2016a, 2017).

Prior to leaving England, Keen prepared for the upcoming challenges of serving a people with which he was unfamiliar, but he was also filled with anticipation of working in a region whose natural history was relatively unexplored. Keen's letters to Albert K. L. G. Günther (1830–1914), Keeper of Zoology at the British Museum (Natural History), London, from 1875 to 1895 (Gunther 1930), now the Natural History Museum (NHM), revealed his anticipation of reaching the Queen Charlotte Islands and the discoveries he felt sure would follow. Shortly before arriving at Masset, Keen wrote to Günther, "As I remember you said that almost anything from [the Queen Charlotte Islands] would be of interest, I shall hope to send you a good deal from time to time" (Keen 1890). Upon his arrival, he re-iterated his awareness of the value of specimens from this new locality, again in a letter to Günther: "The productions of the island, one would think, ought to be of considerable interest as it is separated from the mainland by a channel [Hecate Strait] about 60 miles wide" (Keen 1890). Keen's work on the natural history of the Queen Charlotte Islands had begun, and recognition of the results soon followed.

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In the years that followed, Keen assembled extensive collections of insects and other animals, particularly of mammals, and some plants. Although Keen collected the specimens and made notes on the behaviour and habitat of many of the species (Sealy 2015, 2017), he had to rely on specialists to identify them. Specimens collected at Metlakatla and during the first year or so of service at Masset were sent to Günther at the British Museum (Sealy 2015, 2016c). By late-1892, impatient with delays in receiving determinations from busy curators in England and the irregular mail service, Keen began sending most specimens of insects and a few other invertebrates and mammals to the Central Experimental Farm in Ottawa. There, entomologist James Fletcher (1852–1908) identified some of the insects, but sent the other specimens to specialists in France and the United States. Fletcher, a highly acclaimed entomologist (Gibson and Groh 1909), recognized the seriousness and energy that Keen brought to his observations and collections and, thus, both contributed importantly to knowledge of the natural history in a region that was almost completely unexplored biologically at the time.

Keen's contributions to knowledge of the insect fauna of the coastal region of central British Columbia took three forms: (1) published lists of beetles identified by specialists, frequently annotated with notes on behaviour and seasonal habitats (Sealy 2015); (2) specimens, mostly of beetles, incorporated into catalogues, taxonomic revisions, and distributional works published by other entomologists and in museum reports (e.g., Harrington 1894, Fannin 1898, Kavanaugh 1992); and (3) eight taxa, including two taxa that Hatch (1957b) considered to be *nomen nuda*, described from specimens collected by Keen. These specimens were catalogued in the National Collection of Insects (Ottawa), the Natural History Museum (London, UK), Provincial Museum of British Columbia (now Royal British Columbia Museum, Victoria), and Biological Survey of the U.S. Department of Agriculture (U.S. National Museum, Washington).

Insect taxa named for John H. Keen. The dates of collection of Keen's insect specimens, types and otherwise, are generally not known except for the year or the season ("February", "summer"). It is known with certainty, however, that specimens were collected on the Queen Charlotte Islands between mid-September 1890 and late-1898, during Keen's residency at Masset (Sealy 2016a). Specimens were collected at Metlakatla in June and July 1890, while Keen waited for the steamer to transport him to Masset, and again after settling at Metlakatla in late summer of 1899 (Sealy 2016a). Specimens were collected year-round on the Queen Charlotte Islands, within "a circle of five miles' radius from Massett" (Keen 1895), and at Metlakatla, mainly at settlements along the Nass River (Sealy 2016c). Unlike descriptions of new taxa of mammals from the Queen Charlotte Islands that were based on Keen's specimens, in which each was accompanied by a type specimen and institutional registration number (Sealy 2015, 2017), catalogue numbers of type specimens of insects were generally not available. The new taxa listed below are presented in chronological order of the date of description, with the exception of two species considered by Hatch (1957b) to be nomen nuda, which are presented at the bottom of the list.

Pezomachus keenii (Harrington 1894) (Hymenoptera: Ichneumonidae). Among a collection of several undescribed ichneumonids assembled by the Rev. G. W. Taylor near Victoria, British Columbia, and entrusted to W. H. Harrington, were two new species of wasps collected by Keen near Masset. Regarding the first species, Cremnodes canadensis, Harrington (1894) noted that it was "[d]escribed from one ♀ specimen from Queen Charlotte Islands, sent by the Rev. J. H. Keen to Mr. Fletcher. A very interesting wingless species, with rufous head and abdomen, and testaceous thorax and legs ..." This species now resides in the genus Polyaulon Foerster. It took only seven lines for Harrington (1894) to describe the second species, this one placed in the genus Pezomachus, based on four females collected at Masset by Keen, "... after whom I have much pleasure in naming the species, as a recognition of his efforts to advance our knowledge of the insect fauna of this distant portion of the Dominion." Pezozmachus was synonymized with the genus Gelis by Viereck (1914).

[George W. Taylor (1854–1912) was another among several early clergymen who collected insects in British Columbia. He became a sought-after expert on the Geometridae and exchanged specimens with collectors and identified moths for others (Riegert 1991). William H. Harrington (1852–1918) was one of the founders of the Ottawa Field-Naturalists Club, a still-active organization that publishes the *Canadian Field-Naturalist*. His entomological contributions focused on systematics and economic entomology, particularly of the Hymenoptera and Coleoptera (Gibson 1918).]

Platyceropsis keeni (Casey, 1895) (Coleoptera: Lucanidae). This is the first of three species of beetle that Capt. Thomas L. Casey (1857–1925) named in honour of Keen, based on a single female specimen (Benesh 1946). Casey noted (1895) "This interesting species was discovered by Rev. J. H. Keen [at Masset], and the original specimen kindly given me for description by Mr. [H. F.] Wickham, with permission of Mr. James Fletcher, of Ottawa. It has recently been taken in abundance." This species had been collected only on the Queen Charlotte Islands (Wickham 1899) and, with specimens of Haida keeni (see below), were part of a collection of 141 species of beetle that Keen presented to the British Columbia Provincial Museum in Victoria (now Royal British Columbia Museum). The list of Keen's specimens was included in Fannin's (1898) preliminary catalogue of collections deposited in the Museum. This species belongs to the genus Platyceropsis (Benesh 1946).

[Henry Frederick Wickham (1966–1933), professor of entomology at the State University of Iowa (now Iowa State University), was a specialist in the Coleoptera (Anonymous 1934). He described many new species of beetle, including fossilized species, and was among several specialists to whom James Fletcher forwarded Keen's specimens for identification and whom Keen acknowledged (Keen 1895). Wickham's field experience extended to British Columbia, where he collected insects for one month near Victoria on Vancouver Island in 1889 (Wickham 1890) and, in 1891, collected them in Alaska and the adjacent portions of British Columbia (Wickham 1893).]

Oxypsylla keeni (Baker, 1896) (Siphonaptera: Ceratophyllidae). Keen collected fleas secondarily as they escaped from the pelage of Keen's Mouse (Peromyscus keeni (= Sitomys keeni Rhoads) and from mouse nests, while recording notes on this species' behaviour (Keen 1896; also see Sealy 2015). From "several [male and female] specimens taken on Sitomys keeni at Masset... in August of 1895, by Rev. J. H. Keen", Baker (1896) described a new species, Pulex keeni, and named it in honour of Keen. He also acknowledged his indebtedness to James Fletcher "... for the opportunity of examining this very interesting and well-marked form." Baker (1904) supplemented the original description of Pulex keeni with figures and re-assigned it to the genus Ceratophyllus.

Jordan (1933) re-assigned *Ceratophyllus* to the genus *Opisodasys*. A lectotype male was designated by Smit and Wright (1978), and the species was placed in the subgenus, *Oxypsylla*, by Smit (1983), where it resides today. The preferred host of *Oxypsylla keeni* is the deermouse genus *Peromyscus* in south–central British Columbia, on Vancouver Island and other coastal islands, including Haida Gwaii, and north along the panhandle of Alaska (Holland 1985), as well as deermice throughout the northwest United States, including western Montana and northern Nevada and Utah (Lewis 2008).

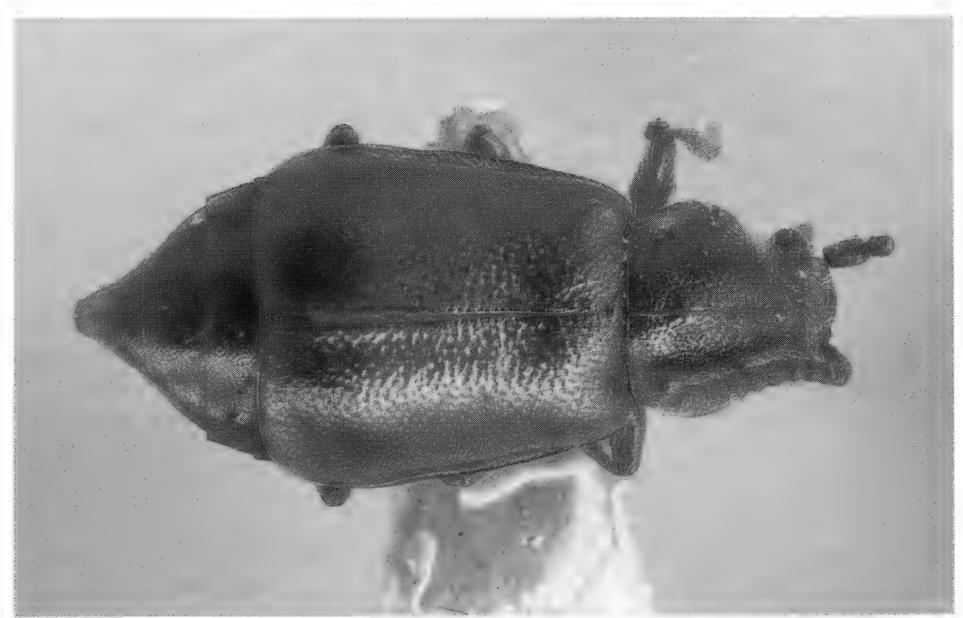
Baker benefited further from Keen's collecting skills, with his description of another new species of flea taken from a deermouse nest at Masset in 1898. Two females provided the basis for Baker's (1898) description of *Typhlopsylla charlottensis*, based primarily on account of its reduced eyes. Further study prompted Baker (1904) to expand "the meagre original description" of this new species, and he re-assigned it to the genus *Ceratophyllus*. Rothschild (1915) later designated the genus as *Catallagia*, thus, *Catallagia charlottensis*.

After a furlough in England, Keen resumed his duties in mid-July 1899, this time at the mission at Metlakatla, where he continued to collect specimens, including fleas. Holland (1985) included two species that included specimens collected by Keen: *Hystrichopsylla occidentalis occidentalis* Holland (from Norway Rat [*Rattus norvegicus*]

at Metlakatla), and *Monopsyllus ciliatus protinus* Jordan (from Red Squirrel [*Tamiasciurus hudsonicus*] at Metlakatla and *Tamiasciurus* sp. at Inverness).

Haida keeni Keen 1897; Brown 1944 (Coleoptera: Staphylinidae). The history surrounding the naming of Haida keeni may be unique. Among Keen's beetle specimens sent by Fletcher to Mons. Albert Fauvel, of Caen, France, a specialist on the Coleoptera (e.g., Fauvel 1889), was a specimen collected at Masset on 18 October 1893 (Figure 1; also figured by Keen (1897) and frontispiece in Campbell 1978: facing p. 1) that was recognized as a new taxon. Fauvel suggested it be named Haida keeni, the genus suggested in honour of the Haida people, the traditional inhabitants of Haida Gwaii with whom Keen was working, and the species after Keen, the collector. But Fauvel never published a formal description of Haida keeni, although he is sometimes named as the author of the genus (e.g., Fannin 1898, but see Armett and Thomas 2000). As Hatch (1957a) noted, this resulted in Keen's (1897) "interesting but taxonomically most inadequate remarks constitut[ing] the original description of both genus and species, and resulted in Keen being in the anomalous position of naming a species after himself!" Forty-five years later, Haida keeni was accurately described by Brown (1944), based on three additional specimens collected at Masset in 1893 and catalogued in the National Insect Collection in Ottawa. But Keen, not Brown, is still recognized as the species' author (e.g., Hatch 1957b, Campbell 1978, Bosquet et al. 2013; also see Hatch 1957a).

Keen (1895) noted this species is "Not common. Found in moss at roots of trees, in December." He did not collect this species at Metlakatla (Keen 1905), although specimens have been taken subsequently from the mainland, from southeast Alaska to southwestern British Columbia (Campbell 1978).



**Figure 1.** Haida keeni registered in the Canadian National Collection of Insects, Ottawa, Ontario, collected by the Rev. J. H. Keen on 18 October 1893 near Masset, Queen Charlotte Islands, British Columbia. Photo credit: Serge Laplante, courtesy of the Canadian National Collection of Insects, Arachnids and Nematodes, Agriculture and Agri-Food Canada, Ottawa.

Atheta (Lamiota) keeni Casey 1910 (Coleoptera: Staphylinidae). Describing this species (type, USNM 38480 insects, collected at Metlakatla), Casey (1910) noted, "This strikingly distinct species is dedicated with pleasure to Rev. J. H. Keen, who has made

many interesting discoveries among the small clavicorn Coleoptera of the northern coast of British Columbia." Considered a valid species, *Atheta keeni* was designated the type species of the subgenus *Lamiota* Casey, although Gusarov (2003), who supplemented Casey's description of *Atheta keeni* with illustrations of body parts, cautioned that the "Subgeneric assignment of *At. keeni* and the status of the name *Lamiota* require further study." *Atheta keeni* is known from Alaska, British Columbia and Oregon (Gusarov 2003).

Gyrophaena keeni Casey, 1911 (Coleoptera: Staphylinidae). The description of the third species of beetle named for Keen by Casey (1911) was accompanied only by a brief acknowledgement and statement of the type locality: "British Columbia (Metlakatla), – Keen." This species, described from a male specimen, and others in the genus Gyrophaena proposed by Casey have been confirmed as valid. Seevers (1951) grouped five closely related species of Gyrophaena in a "Keeni group" (also see Stace Smith 1957), which is composed of fungus-feeding beetles, obligatory inhabitants of the fungi during the larval and adult stages.

Bryobiotos keeni Fauvel (Coleoptera: Staphylinidae). Hatch (1957b) considered this species to be a nomen nudum, and thus not described and not valid. Keen (1895) noted in the entry for this species in his list of beetles from Masset that it was "Occasional in June under stones on sandy beach, between tide marks. Larvae in same place."

Anthobium keeni (Fauvel) (Coleoptera: Staphylinidae). Keen (1895) listed this species in the genus Lithrimaeum, noting "Several in rotten sea-weed, in June", but Hatch (1957b) considered it to be a nomen nudum. Among the locations given by Hatch (1957b) in the description of his new species, Anthobium sinuosum, was Metlakatla, where one of the female paratypes was collected, probably by Keen. It is possible that keeni was placed with the new species.

#### **CONCLUSIONS**

Keen's collecting career in coastal British Columbia spanned more than 20 years. His last specimen apparently was a sap-feeding beetle (Fabogethes nigrescens (Stephens)) taken at Metlakatla in 1915 and catalogued in the British Museum (Easton 1955, also see Hatch 1957b). Osgood (1901), in the first treatise of the fauna and flora of the Queen Charlotte Islands, extolled Keen's dedication and far-reaching contributions to natural history, acknowledging that the little that was known of the vertebrate fauna of the islands "was entirely due to the zeal of Rev. J. H. Keen ..." Accolades from entomologists followed. Baker (1904) stated "All of our records [of fleas] for the Queen Charlotte Islands are due to this gentleman, and his contributions have been most important ones." Riegert (1991), in a brief sketch of Keen's life and accomplishments, noted that "Our knowledge of the original beetle fauna of the northwest B.C. coast is due primarily to the painstaking and energetic collecting of this remarkable clergyman." This sentiment was echoed by Kavanaugh (1992) who acknowledged that "Development of our present knowledge of the carabid beetle fauna of the Queen Charlotte Islands began with the Reverend J. H. Keen, Anglican missionary to the Haida people ..." In a memoir published following Keen's death in England in 1950 at the age of 98, Hatch (1957a) acknowledged that entomologists were aware of Keen's "short series of papers" on the beetles of the Queen Charlotte Islands, but he lamented that there was a "... complete dearth of published information about Rev. Keen." Hearne (1997) extended Hatch's (1957a) brief biography of Keen in a tribute to the "forgotten naturalist" of the Queen Charlotte Islands. Recently, photographs of Rev. Keen have been uncovered (Sealy 2016a).

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# Western balsam bark beetle, *Dryocoetes confusus* Swaine (Coleoptera: Curculionidae: Scolytinae), *in situ* development and seasonal flight periodicity in southern British Columbia

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#### **ABSTRACT**

In situ development and seasonal flight periodicity of the western balsam bark beetle, Dryocoetes confusus Swaine, was observed in subalpine fir, Abies lasiocarpa (Hook) Nutt. stands in southern British Columbia for three years between 1998 and 2002. This study shows developmental differences of western balsam bark beetle in downed and in standing, live subalpine fir. Larval development was slower in the downed trees. Recorded daily minimum phloem temperatures were significantly lower for downed trees than for standing trees during periods of beetle development and flight. There were no significant differences in the recorded daily maximum phloem temperatures between standing and downed trees until late summer, when downed trees saw cooler daily maximum phloem temperatures. This cooler host habitat would provide fewer degree days for insect development. Three distinct larval instars were identified by head capsule measurement. There were two flights per season, the first and major flight occurring from late June to late July, and the other smaller flight occurring in late August. A combination of minimum daily phloem temperatures reaching 5° C and maximum daily phloem temperatures approaching 20° C appeared to trigger the onset of beetle flight, with flight initiated earlier in the season at lower elevations.

Key words: development, instar determination, subcortical temperature

#### INTRODUCTION

The western balsam bark beetle, Dryocoetes confusus Swaine (Coleoptera: Curculionidae: Scolytinae), is the most destructive insect pest of mature and over-mature subalpine fir, Abies lasiocarpa (Hook.) Nutt. in British Columbia (B.C.) (Garbutt 1992). Western balsam bark beetle is found throughout the range of subalpine fir and is the dominant successional force in high-elevation ecosystems of the Engelmann Spruce-Subalpine Fir zone (ESSF) (Stock et al. 1994; Maclauchlan 2016), which include dry, moist, and wet subzones (Meidinger and Pojar 1991). Tree mortality from this beetle is first noticed in stands approaching 70–90 years of age (Maclauchlan 2001) and, as stands age, the aggregated pattern of attack by western balsam bark beetle describes the smallscale gap dynamic process, which over time releases the next generation of subalpine fir (Stock et al. 1994). Subalpine fir is susceptible to a wide variety of other disturbance agents, including two-year-cycle budworm, Choristoneura biennis Freeman, various root and butt rots, stem rots, animal damage and windthrow (Alexander 1987; Unger 1995; Parish and Antos 2002). Fire is relatively rare in the wetter ESSF subzones (Anon. 1995), often seen as small, localized events. Despite these other disturbances, western balsam bark beetle is one of the primary drivers of succession in both the ESSF and other subalpine fir-dominated ecosystems throughout B.C. (Maclauchlan et al. 2003; Maclauchlan 2016).

Western balsam bark beetle selectively kills small groups of subalpine fir at a relatively low, but constant, level each year in infested stands (Stock et al. 1994; Unger

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and Stewart 1992; McMillin et al. 2003). Recently killed, red trees occur in groups of two or more, often spread over hundreds of hectares (Stock 1991). Attack rates of western balsam bark beetle in southern B.C. can range from 0.7 to 1.6% of subalpine fir annually in a stand, depending upon ecosystem (Maclauchlan 2016). The beetle attacks large-diameter, standing live trees and downed subalpine fir. Western balsam bark beetle consistently attacks trees from the largest-diameter classes in each stand, but the mean diameter of attacked trees between sites may vary significantly (ranging from 10 cm diameter at breast height to over 50 cm), indicating that factors other than diameter contribute to the susceptibility of subalpine fir to western balsam bark beetle (Bleiker et al. 2003). Susceptibility has been associated with tree diameter, age, recent radial growth, and induced resinosis (Bleiker et al. 2003). Although cumulative mortality can reach significant levels in chronically infested stands (Garbutt and Stewart 1991; Maclauchlan 2016), western balsam bark beetle may be less aggressive or exhibit unique attack dynamics compared to other tree-killing bark beetles at epidemic levels. Beetle populations persist within a stand for many years until most of the mature subalpine firs are killed (Garbutt 1992; McMillin et al. 2003). This selective and patchy distribution of mortality suggests that western balsam bark beetle may be limited by the abundance and distribution of susceptible hosts, as well as the harsh environment in which they live.

Many researchers (Hansen 1996; Gibson et al. 1997; Negrón and Popp 2009; Stock et al. 2013) have concentrated primarily on the flight periodicity and insect activity within subalpine fir stands. A paucity of work has been done on life-stage development of the western balsam bark beetle primarily due to the remote nature of most subalpine fir forests. In B.C., adults generally emerge in late June and fly until late July, locating suitable host trees through kairomones and primary attraction, at which point the males initiate construction of nuptial chambers beneath the bark (Bright 1976; Garbutt 1992; Stock et al. 2013). The species is polygamous, with males often attracting three or more females in a nuptial chamber. Females mate, then lay eggs in brood galleries that radiate out from the nuptial chamber, and toward the end of August will construct feeding and hibernation niches (Bright 1976) for the winter. Mature females may resume laying eggs the following year within hosts that have adequate phloem resource, whereas females within trees that are fully occupied with brood may emerge mid-summer to locate new hosts (Bright 1976). In addition to the main attack flight, comprised of males and females, an additional smaller flight, largely comprised of parent females (Hansen 1996; Gibson et al. 1997; Stock et al. 2013), has been observed later in the summer. In this lateseason second flight, the females join existing gallery systems and often create hibernation niches.

Mathers (1931) first described the life cycle of the western balsam bark beetle in B.C., demonstrating that it completed its life cycle within two years. Bright (1963) subsequently speculated that the insect might be capable of completing its life cycle in only one year in the western and southwestern United States. Whether the brood is capable of developing to the adult stage in one year has not been shown. Insects have different strategies to cope with fluctuating weather conditions and phases of growth and dormancy: some undergo hormonally controlled diapause (obligatory diapause) that prevents insect development even when environmental conditions are good (Gilbert 1990), while others only undergo diapause when induced environmentally (facultative diapause). The spruce beetle, Dendroctonus rufipennis Kirby, is known to have a life cycle that can vary in duration, from one year up to three years, depending on climatic conditions and suitability of host material available (Knight 1961; Schmid and Frye 1977; Hansen et al. 2001; Bentz et al. 2010). Johansson et al. (1994) describe a flexible generation time for Dryocoetes autographus (Ratz.), a circumpolar species, that has expanded its range in Norway north, with the establishment of its host species, spruce. Swift and Ran (2013) noted that climate change may have a pronounced effect on highelevation forests and associated insects. Therefore, a greater understanding of the developmental requirements for western balsam bark beetle is needed.

This study focuses on western balsam bark beetle life-stage development in standing and down subalpine firs, and flight periodicity over a range of elevations in southern B.C. We also studied the relationship of temperature to western balsam bark beetle development in standing and down host material. These trials were conducted in 1998, 1999, and 2002 at various field sites in southern B.C.

#### MATERIALS AND METHODS

The developmental biology of western balsam bark beetle was investigated in the field by flight periodicity trapping, sampling of *in situ* life stages, and weather monitoring. These studies focused on the two very different stages in the beetles' life history: 1) emergence and flight dispersal and, 2) brood production and maturation within the host tree. On-site weather monitoring was used to determine critical threshold subcortical (phloem) temperatures for flight and development.

Seven field sites were selected throughout the southern interior of B.C. in subalpine fir ecosystems with active populations of western balsam bark beetle. Two sites, Cherry Ridge and Sun Peaks, were used in all three studies, while the other sites were used for monitoring flight dispersal timing only (Table 1).

One micro-logger-based climate station (Campbell Scientific Inc.) was set up at each of the Sun Peaks and Cherry Ridge sites to monitor ambient, duff and phloem temperature of western balsam bark beetle-attacked trees. The Sun Peaks and Cherry Ridge climate stations were located on the north aspects of standing trees within close proximity (±10 m) of other attacked trees, where flight dispersal monitoring and detailed life-stage studies were conducted. The Sun Peaks climate station was set up on June 18, 1998, and again on June 15, 1999. The climate station was installed next to a standing tree, and thermocouples were inserted three meters up the bole in the phloem of five nearby attacked subalpine fir. Thermocouples were inserted into western balsam bark beetle entrance holes. Thermocouples were also placed in the phloem on downed trees. Ambient air temperature was recorded at the relative humidity sensor on the climate station. Another climate station was installed on June 16, 1999, at Cherry Ridge, as per Sun Peaks. Numerous variables were recorded, but only date and phloem temperature were used for analyses in this study. On August 5, 1999, the climate station at Cherry Ridge malfunctioned, and no further weather data were recorded.

In 1998, three 8-funnel (8 plastic funnels aligned vertically over each other) Lindgren multiple-funnel traps (Lindgren 1983) were erected between June 18 and August 31 at Sun Peaks and monitored regularly for western balsam bark beetle flight activity. Traps were placed along an elevation gradient (1,450 m; 1,650 m; 1,850 m), and all traps were positioned just inside the stand edge. Each trap was hung on an aluminum pole, with the top of the trap approximately two meters above the ground. Traps were baited with the commercially available (±)-exo-brevicomin (release rate 0.4mg/24 h) bait for western balsam bark beetle (supplied by Phero Tech Inc., Delta, B.C., Canada, and now available through Distributions Solida Inc., Scotts Miracle-Gro Company).

In 1999, trapping trials were established at Sun Peaks and Cherry Ridge sites to follow the flight timing and activity of western balsam bark beetle. Four traps were hung at Sun Peaks over an elevational range at 100–150 m intervals (1,450 m–1,850 m), and two traps were hung at 1,650 m at Cherry Ridge. Weekly trap catches were collected from June 16 to October 9 at Sun Peaks. At Cherry Ridge, collections were made at irregular intervals from June 15 to October 30.

In 2002, a more comprehensive trapping trial was conducted, using nine sites (three traps per site) in six geographic locations (Table 1) within four ESSF subzones. Lindgren funnel traps were set up in a triangular formation, with the traps being approximately 20 metres apart. Traps were established from May 29 to June 21, with regular collections beginning June 19 until September 27. During the peak flight period, trap collections were made more frequently, up to three times per week, until the peak flight was over.

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Cherry Ridge	ESSFwc	formund Simund	5573800	394250	1,650	1999	1999	1999
Spius Creek	ESSFmw		5539281	633130	1,470-1,635		2002	
Torrent Creek	ESSFwc	generally.	5603075	387147	.,685		2002	
Apex Mountain	ESSFxc	Same and	5478306	289090	1,670-1,900		2002	
Buck Mountain	ESSFxc	Secured Secured	5549029	360134	1,750		2002	
Sunset Main	ESSFxc	granned	5527100	700200	1,820		2002	

<sup>a</sup> ESSF subzone descriptions: dc=dry, cold; wc=wet, cold; mw=moist, warm; and xc=very dry, cold.

All insects collected from the trapping trials were stored in zip-lock bags, labeled, and frozen until processed in the laboratory, where each sample collection was then counted and the western balsam bark beetles were sexed.

Trap catch results were compared to daily weather patterns at sites with climate stations. The maximum and minimum phloem temperatures were plotted against trap catch to interpret the relationship between insect flight and subcortical temperature.

The literature describes western balsam bark beetle as having a two-year life cycle (Mathers 1931; Bright 1976). To capture all life stages (from initial attack through newly emerged adults), sample trees meeting specific criteria were selected in 1999 at the Sun Peaks and Cherry Ridge study sites. External signs and symptoms, such as foliage fade, and presence of entrance holes and frass on the bole, were used to select candidate trees and ascertain year of attack. Western balsam bark beetle tree baits ((±)-exo-brevicomin; release rate 0.4mg/24 h) were attached to two standing live and two freshly felled subalpine firs in early June 1999 to induce western balsam bark beetle attack. Downed trees were felled into the stand, where they were well shaded, and no limbs were removed. Both standing and down (natural blowdown) subalpine fir attacked by western balsam bark beetle in 1997 or 1998 were selected for sampling in 1999 to determine if there were obvious developmental differences between these two host scenarios. Six trees at Sun Peaks and three trees at Cherry Ridge were suitable for sampling (Table 2). All sample trees were in close proximity ( $\pm 10$  m) to the climate stations. Sampling took place at weekly intervals between June 15 and September 20, 1999. A rigorous sampling procedure was followed at each sampling date to help interpret progression of attack and tree symptoms. For each sample tree, numerous foliar attributes and bole symptoms were recorded, but only the ones used in the analysis are described. In some cases, particularly in the trees that were attacked in 1997, a comment was made at each dissection as to the abundance of exit holes. A ladder was used to access western balsam bark beetle attack found higher on the bole, up to 3.5 meters. A 20-cm x 20-cm template was centered over an entrance hole, and the bark was carefully removed to expose the gallery system. Gallery systems (Figure 1) were described to help elucidate the stage in the life cycle and productivity. All western balsam bark beetle life stages present (eggs, larvae, pupae, and parent and teneral adults) were collected and placed in vials of 70% ethanol for future processing in the laboratory.

Head capsule measurement is a commonly used method to determine the instar of immature insects (Bleiker and Régnière 2014). The head capsules of all western balsam bark beetle larvae collected in the field were measured using a dissecting microscope. Every measurement was taken at 4.5 X magnification, which yielded a micrometre measurement of 0.022 mm. Measurements were taken across the widest portion of the sclerotized head capsule. All head capsule measurements were sorted in ascending order and plotted to display frequency distribution. The lowest frequency class between peaks on a histogram can be used as the cut-points for each instar and are often visually determined (Logan *et al.* 1998; Bleiker and Régnière 2014). From these frequency distributions, delineation of instars was determined by visually identifying cut-points. Each instar was assigned a head capsule size range. All larvae collected were then given an instar designation and these data were then compared to field temperatures and date of field sampling. These comparisons provided the interpretations of life-stage occurrence and duration in standing and down trees.

#### **RESULTS**

Very few beetles were caught in 1998 at the Sun Peaks site (170 beetles over 75 days), with the highest trap catches occurring July 6 and July 10. In 1999, at the Sun Peaks site, peak trap catch occurred between July 27 and August 8, with 64 beetles collected July 27 and 40 beetles collected August 8. There were additional small trap catches until early September (158 beetles in total). In 1999, at the Cherry Ridge site, 605

western balsam bark beetles were caught in two funnel traps, with the maximum number of beetles collected on August 3, similar in timing to insects collected at the Sun Peaks.

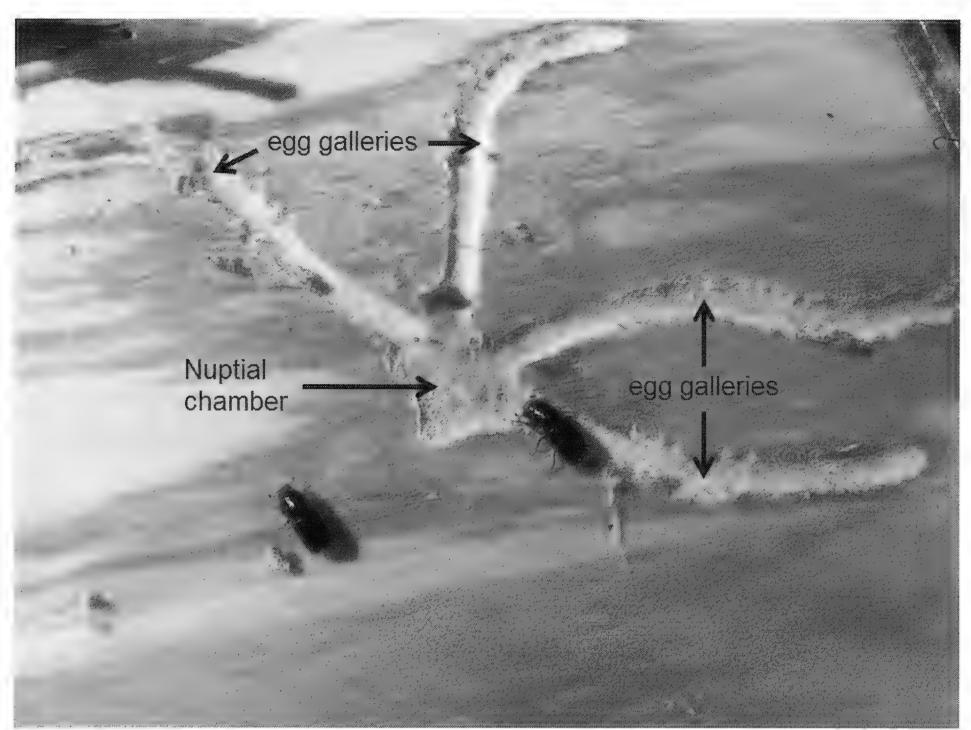


Figure 1. Photograph of a western balsam bark beetle gallery system, showing central nuptial chamber, four egg galleries and two parent females.

In 2002, insects were collected regularly from six additional sites, then counted and sexed (Figure 2). In total, in 2002, 348 trap samples at nine sites were collected and assessed, with 4,897 western balsam bark beetles caught from June 24 to September 10. Significantly (t test, p<0.05) more males than females were trapped early in the flight season (Figure 2). After July 9, 2002, equal or fewer male than female beetles were trapped.

Western balsam bark beetles were collected from traps beginning in mid- to late June, with the exception of one high-elevation site at Spius Creek, west of Merritt, B.C., where no beetles were collected until mid-July (Figure 3). At Sun Peaks, where there were three trapping sites on an elevational gradient, beetle flight occurred earlier at the lower-elevation site, gradually increasing in insect numbers later in the season at the higher-elevation site. Smaller numbers of beetles were caught at the high-elevation site at the onset of the flight period, with the majority trapped from July 9–20 (Figure 3). Although sites at or above 1,600 meters in elevation had high trap catches (Figure 3), there was no significant difference (p≥0.05) in mean trap catch numbers at the different elevations (1,450 m−1,600 m; 1,600 m−1,750 m; 1,750–1,900 m). There was no significant difference in trap catch numbers between the ESSF subzones, where the traps were located.

A second lesser flight beginning in late August was evident at Spius Creek, Torrent Creek, Sun Peaks, and Apex in 2002. At Sun Peaks, beetles were caught in varying numbers throughout the main flight period and into the second. Beetles were trapped in high numbers at all three Sun Peaks sites, with the highest catch at the mid-elevation site

(1,535 m) from June 24 to July 9. An elevational cline was observed at Sun Peaks, with most beetles caught in the upper-elevation site (1,850 m) (July 9 through July 19), when trap catches at the mid- and low-elevations sites were declining (Figure 3). This second flight was small and comprised approximately 7% of the total number of insects trapped at all study sites.

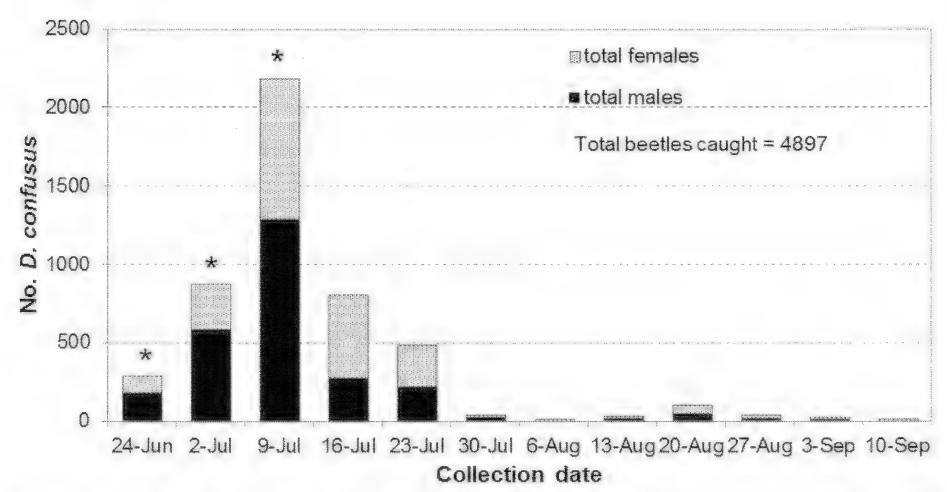


Figure 2. Total number of western balsam bark beetles collected during the 2002 flight period from 348 traps at nine sites in the southern interior of B.C., from June 24 to September 10. Asterisk above bars indicates significantly (t test, p<0.05) more males than females were trapped. Total number beetles caught = 4897.

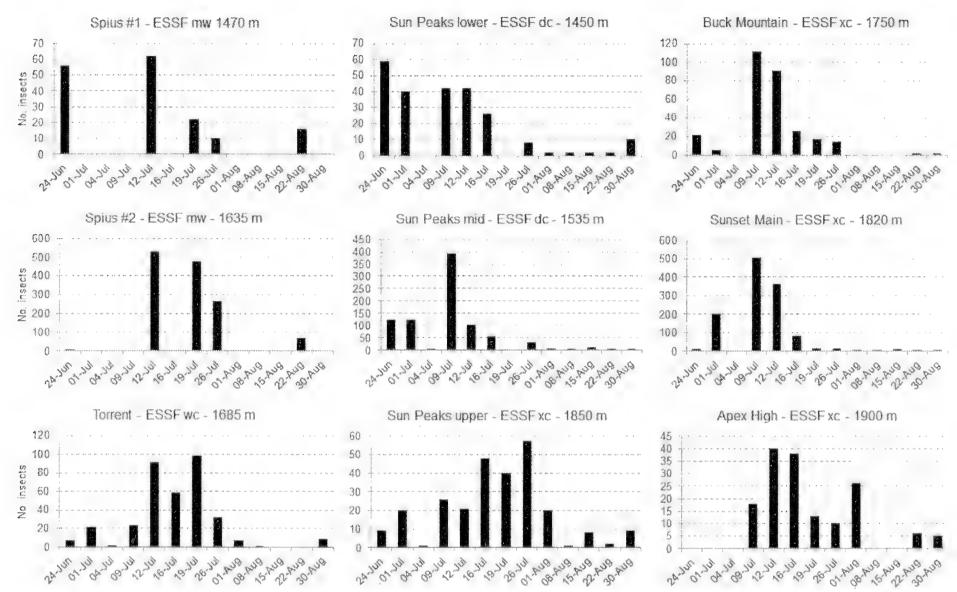
Not one of the four baited subalpine fir trees was successfully attacked in 1999. Numerous beetles had initiated attack on the baited trees, as evidenced by the presence of entrance holes and frass. However, minimal egg galleries and brood were found in samples. From the nine non-baited trees attacked in 1997 and 1998, 7,257 life stages were dissected and preserved for further study in the laboratory (Table 2).

Head capsule sizes ranged from 0.276 mm to 1.058 mm wide. Three distinct peaks emerged from the measurements. First instar larvae ranged from 0.276 mm to 0.437 mm  $(0.379 \pm 0.005 \text{ mm}, \text{ mean} \pm \text{S.E.})$ ; second instar larvae from 0.460 mm to 0.644 mm  $(0.549 \pm 0.002 \text{ mm})$ ; and third instar larvae from 0.667 mm to 1.035 mm  $(0.826 \pm 0.001 \text{ mm})$ , with only two larvae with head capsules measuring 1.058 mm. Our data clearly show three distinct larval instars based on head capsule size frequency distribution, and there are not enough individuals from this study to confirm the possibility of a fourth instar. Figure 4 illustrates the distribution of head capsule widths for larvae collected from the attacked sample trees at Cherry Ridge and Sun Peaks sites. In total, 5,052 western balsam bark beetle larval head capsule widths were measured.

A summary of life stages found in 1999 from subalpine fir attacked in 1997 and 1998, at Sun Peaks and Cherry Ridge, are presented as proportional data in Table 2. Although life stages were dissected out of and counted from the 1997-attacked trees at Sun Peaks, new adults had emerged prior to the onset of sampling. Therefore, emergence holes were noted, but exact counts were not possible at that time.

In 1999, at Cherry Ridge, 3,442 life stages were dissected from three trees attacked in 1998. Two trees were standing attack, while the third tree was down on the ground. Although the two standing trees contained variable numbers of beetles, the proportion of each life stage dissected from the trees was similar (Table 2). This was in contrast to the life stages dissected from the downed attacked subalpine fir, where, by the end of the summer, only 4.5% of the insects dissected from this tree were teneral adults, compared

to 18.3% and 21.7% of those dissected from the standing attacked trees. A similar pattern existed in the 1998-attacked trees from Sun Peaks, where development was slower and a smaller proportion of insects reached the teneral adult stage in the attacked downed trees (Table 2, Figure 5).



**Figure 3.** Comparison of western balsam bark beetle trap catches at nine sites in the southern interior of B.C. (2002) from June 24 to August 30. Number of beetles caught (N) is shown for each location.

There was a demarcated transition between life stages in all three standing trees shown in Figure 5. Late-instar larvae were present June through mid-July, followed by a 3–4 week transition to pupae. At the end of August and into September, the majority of life stages identified were teneral adults. In contrast, brood in down trees shown in Figure 5 displayed much slower development. Late-instar larvae were the predominant life stage found throughout August in downed trees. The transition from larvae to teneral adults was much slower in downed trees than in standing trees (Figure 5).

Seasonal temperature data collected from the phloem of trees by climate stations at Sun Peaks and Cherry Ridge in 1998–1999 were summarized into hourly and daily minimums, maximums and averages. The Sun Peaks climate station malfunctioned in 1999, therefore temperature data were not collected until July 25. Figure 6 shows minimum and maximum daily phloem temperatures at Cherry Ridge from May 20 to August 31, 1999. The recorded temperatures were similar until early July, when the minimum temperatures collected from the phloem of standing trees became significantly higher (t-test p<0.05) than the phloem temperatures recorded on the downed tree. No beetles were caught in baited traps until the minimum daily temperature in the phloem reached approximately 5° C. The same trend was observed from temperature data collected from Sun Peaks. The difference in minimum daily phloem temperatures between standing and downed trees was greater than the difference in maximum daily phloem temperatures on standing and downed trees. Only in mid- to late August did maximum daily phloem temperatures diverge significantly between standing and downed trees (Figure 6).

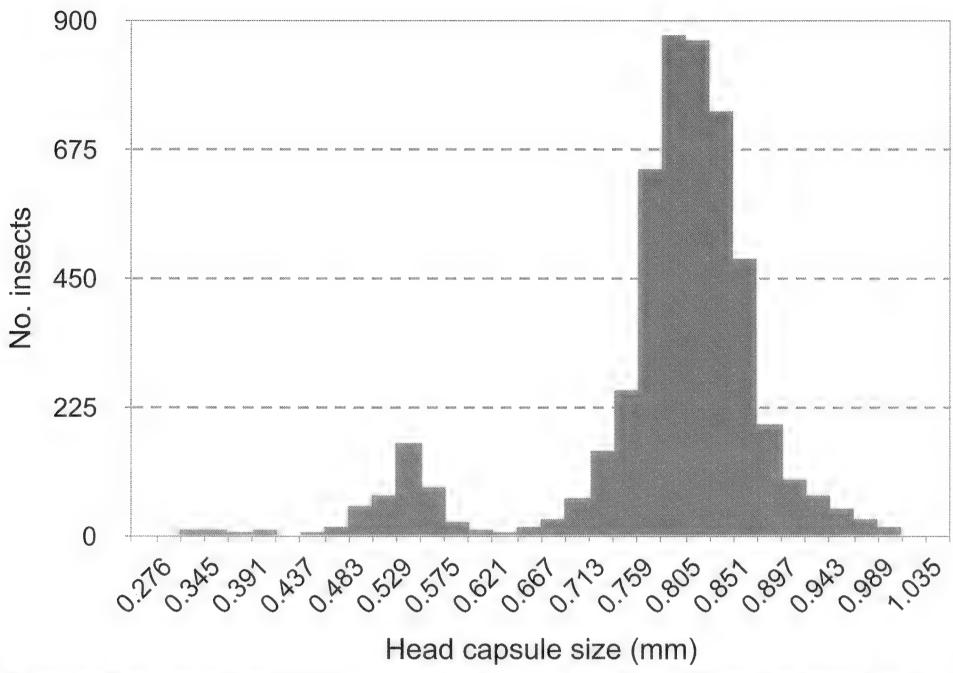
Description of subalpine firs sampled at Sun Peaks and Cherry Ridge in 1999 and summary of proportional life stages present. Year of attack was noted for each sample tree.

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THE REAL PROPERTY OF THE PROPE	1,535	1997-1998	Down-2	310			6.9	26.5	8.7
	1,850	· Problemsky Vision absorbers American in American and the American and th	Down-3	1,800	C;		57.8	22.1	0.7
Cherry Ridge °	1,650	1998	Standing-4	1,437		0.5	56.9	24.4	18.3
THE TAX TO THE TAX THE	1,650	Annual stands that the same th	Standing-5	502	7.0	4.	52.6	25.1	21.7
ARTHUR TO THE	059 <b>*</b> I	Annaly describes and described and an annaly one on a construction of the construction	Down-4	1,503	0	0.5	82.2	80	4. 3.

<sup>a</sup> Some new adults (tenerals) had emerged prior to the onset of sampling as evidenced by the presence of exit holes. Relative abundance of exit holes was noted, but not counted at the time of dissection.

Sun Peaks sampling dates: June 15, 22, 28, July 6, 13, 20, 29, August 3, 10 16, 26, and September 3, 7, 13, 20, 1999.
 Cherry Ridge sampling dates: June 16, 24, July 2, 9, 15, 22, 27, August 5, 11, 20, 23, and September 2, 7, 13, 20, 1999.

Some early flight of western balsam bark beetle at Cherry Ridge may have been missed due to traps not being in place until June 15, 1999. Sustained catches of western balsam bark beetle occurred from early July through September. The combination of exceeding a 5° C subcortical (phloem) daily minimum temperature and reaching or exceeding 20° C subcortical maximum temperature appeared to initiate beetle emergence and flight. There may have been minimal flight prior to trap placement when both these parameters were met (Figure 6; June 10–June 17). Trap data from sites of similar elevation did not have significant trap catches prior to July.

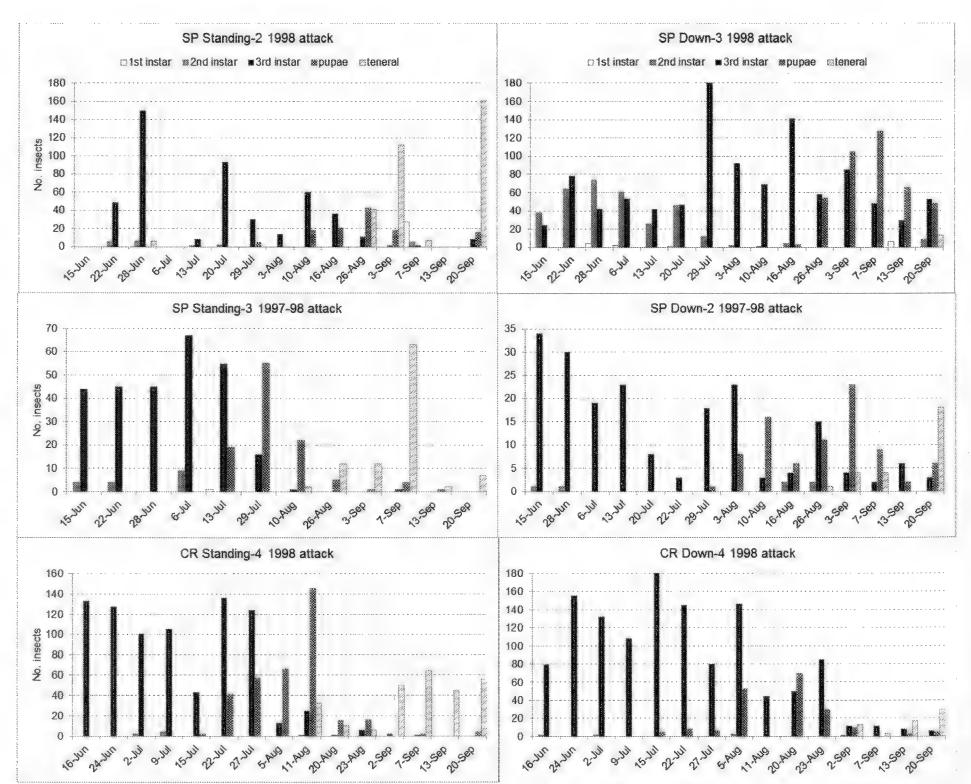


**Figure 4.** Frequency distribution histogram and estimation of larval instar for western balsam bark beetle. A total of 5,052 head capsules were measured.

#### **DISCUSSION**

Our study confirmed the primary flight of western balsam bark beetle occurred from late June through July, depending on site and elevation (Hansen 1996; Gibson et al. 1997; McMillin et al. 2001; Negrón and Popp 2009; Stock et al. 2013). A much smaller, secondary flight occurred later in the season, comprised primarily of females re-emerging from the original host, either to initiate new brood galleries or to create hibernation niches (Maclauchlan, personal observations). Mathers (1931) reported a secondary flight occurring in mid- to late July; however, at the elevations monitored in this study, we only saw this flight beginning in mid-August. Others have also reported a later start to the secondary flight (Hansen 1996; Gibson et al. 1997; McMillin et al. 2001; Negrón and Popp 2009; Stock et al. 2013). Bark beetle flight and development are highly responsive to temperature (Hansen et al. 2001; Gaylord et al. 2008) and latitude (Williams et al. 2008; Bleiker and van Hezewijk 2016). Mathers' (1931) sites were at more northerly latitudes than our study areas, which could explain the difference in flight periodicity. Our results show that the western balsam bark beetle is flexible and may initiate flight earlier if weather conditions support beetle activity. This was seen at the Sun Peaks site, where beetle flight was initiated two weeks earlier in 1998 than in 1999. The former year was a record year for high temperatures in southern B.C. and across Canada

(Environment and Climate Change Canada, Government of Canada. Canada's Top Ten Weather Stories of 1998 (<a href="https://ec.gc.ca/meteo-weather/default.asp?">https://ec.gc.ca/meteo-weather/default.asp?</a> lang=En&n=3DED7A35-1 Accessed on April 7, 2017).

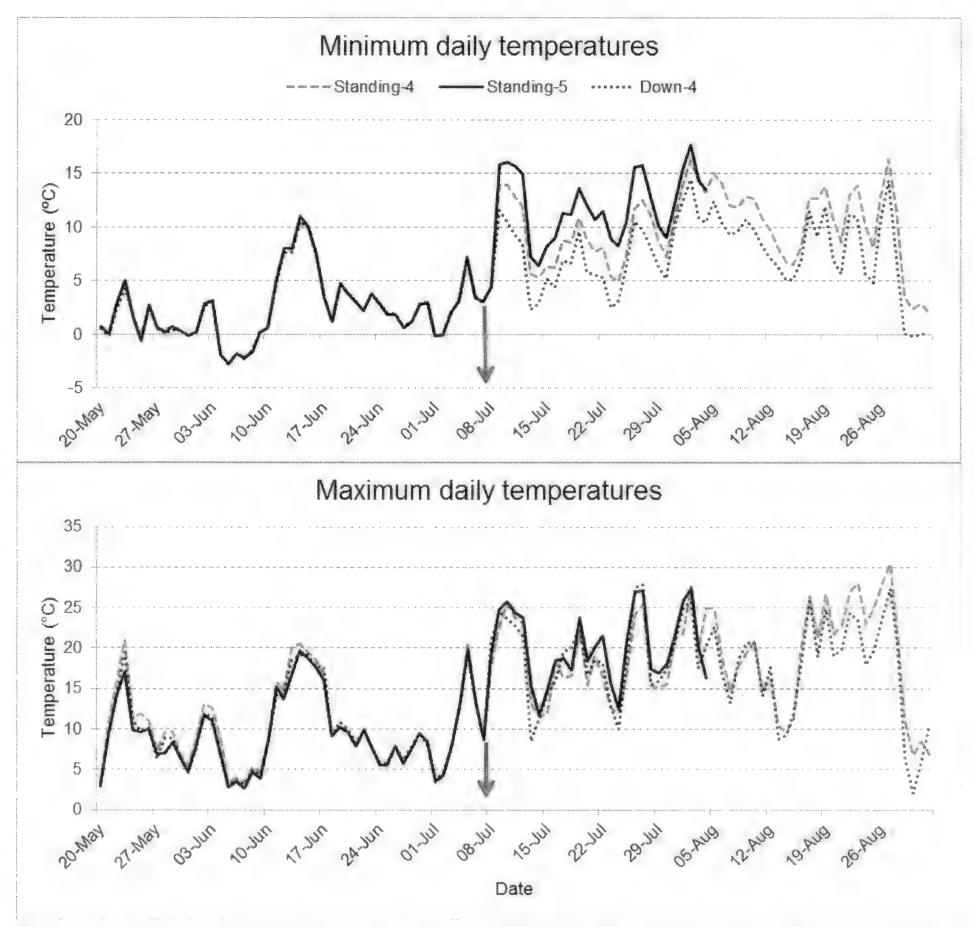


**Figure 5.** Western balsam bark beetle life stages dissected in 1999 from six 1998-attacked subalpine fir at Sun Peaks (SP) and Cherry Ridge (CR). Each sample measured 20 cm x 20 cm and was centered over an entrance hole.

Beetles generally initiated flight once minimum daily phloem temperatures reached or exceeded 5° C and maximum daily phloem temperatures approached 20° C or greater. Maximum daily ambient temperature as a threshold for flight in bark beetles has been determined in several studies (Stock 1991; Hansen 1996; Gaylord *et al.* 2008), and our determination of given site parameters is well within previously published data. Our observations and data clearly indicate that both minimum and maximum phloem temperatures are critical components to western balsam bark beetle flight activity and play an integral role in initiating emergence, flight and dispersal, as well as being an important factor in physiological development.

Subcortical, or phloem, temperature is likely an important factor for insect development. Western balsam bark beetle is active under the bark very early in the season, as evidenced by sawdust and frass being pushed out of entrance holes and movement of life stages under the bark when excised (Maclauchlan, personal observations). This activity has been observed as early as April, with flight not occurring for another two or more months (Maclauchlan, personal observations). This early activity highlights the fact that the physiological development of western balsam bark beetle proceeds within very different temperature limits than is required for flight initiation. Early season subcortical activity may allow female beetles from the late-season flight to

initiate brood production very early the following summer, allowing brood increased developmental time. With the early onset of warm weather in spring and often long, extended summers, this potentially gives western balsam bark beetle better developmental conditions and ultimately could provide an opportunity to shorten their life history to one year, as suggested by Bright (1963).



**Figure 6.** Minimum and maximum temperatures collected from thermocouples inserted in the phloem on three sample trees at Cherry Ridge research site, between May 20 and August 31, 1999. Arrow indicates date of first trap catch (July 8). Traps were established June 15 and checked weekly.

The field collections confirmed three distinct larval instars based upon their respective head capsule size range. The head capsule size range and average we found for first and second instar larvae fall within the range found by Stock (1981). However, our data do not clearly indicate a fourth instar; whereas Stock (1981) detected two overlapping head capsule populations, that he identified as third and fourth instars. Several larvae from controlled temperatures rearing conducted by the authors (unpublished data) had very large head capsules (greater than 1.035 mm), hinting at the possibility of a fourth instar; however, it was an uncommon occurrence in the field. The occurrence of fourth-instar larvae was much more prevalent in a controlled temperature setting (unpublished data) than in our field collections of life stages. Stock's (1981) data

were also obtained from controlled temperatures rearing. Our field data suggest that diurnal fluctuations of subcortical temperature influence physiological development and may prevent a final molt to fourth instar. Late-instar larvae often have higher temperature thresholds for development than early instars do, preventing progression to cold-susceptible advanced life stages before the onset of winter (Safranyik and Wilson 2006). Under field conditions, the western balsam bark beetle may not have an obligate fourth instar.

Our results clearly elucidate that western balsam bark beetle developed more slowly in attacked down trees than in attacked standing trees. We hypothesize that this is due to host finding, host suitability, and a number of climatic factors. Host suitability and attack success may depend on timing of the tree falling (seasoning) (Dyer 1967), placement of the tree on the ground (touching the ground or somewhat elevated), and amount of shade or direct sunlight on the bole (Schmid and Frye 1977) that could affect heat sums needed for beetle development. Blowdown events are relatively uncommon in subalpine fir stands, unlike the regular and often large-scale blowdown events seen in mature spruce stands (Woods et al. 2010). Thus, encountering downed trees is a relatively rare event for this bark beetle, and its search patterns for down material may not be as discerning as for vertical hosts. The highest frequency of blowdown is seen at stand edges (e.g., clearcut edges) and in natural openings (personal observations). Stand edges or more open stand scenarios might afford better ambient conditions for beetle development. Although downed trees may offer the beetles more moderated conditions in late fall through early spring due to the protective insulating characteristics of snow cover, this same snow cover is often retained longer into the spring, depending upon log placement in a stand, thereby keeping logs cool and delaying the onset of beetle development. Also, cooler temperatures in downed trees occur earlier in late summer than in standing trees (Figure 6). Although a good resource from the point of view that the beetles encounter little or no host resistance, it appears that beetle development is prolonged, potentially increasing vulnerability to parasites, predators, woodborer activity, and host deterioration.

Proportionally, four to five times the number of brood in standing trees attained the teneral adult stage prior to winter, compared to down trees at both sites. By the end of summer, less than 10% of insects dissected from 1998-attacked downed trees had reached the teneral adult stage. There was no indication that attack density differed between standing and downed trees (Table 2) (Maclauchlan 2003), and large numbers of larvae developed in the downed trees. However, the cooler and shorter season available to beetles in downed trees suggest they are less suitable hosts for western balsam bark beetle.

Both standing and downed subalpine firs were baited in 1999 to induce attack. Although beetles initially responded to the bait and began excavating nuptial chambers, the weekly sampling demonstrated that none of the trees was successfully mass attacked. This has been observed in the past, where trees baited for western balsam bark beetle, both standing and down, have high levels of unsuccessful attack, compared to natural attacks occurring in close proximity (Maclauchlan *et al.* 2003; personal observation). Bleiker *et al.* (2003) determined that western balsam bark beetle had discerning host-selection capabilities, and parameters associated with host quality that the beetles are able to detect may assist in subsequent developmental success.

Our results clearly revealed a two-year life cycle for western balsam bark beetle in southern B.C. Additional work is needed to determine if western balsam bark beetle larvae or teneral adults require a cold period, or if they can undergo continuous development, as seen in the spruce beetle (Schmidt and Frye 1977), if growing seasons lengthen. The presence of fourth-instar larvae in laboratory rearing suggests that a cold period may be needed or conversely that western balsam bark beetle has evolved a mechanism to postpone the pupation or eclosion process until warmer temperatures trigger it (Johansson *et al.* 1994). Information on the response of this insect to changing

habitat conditions as our high-elevation forest habitats continue to warm may be useful in future forest planning, harvest and management.

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# An updated and annotated checklist of the thick-headed flies (Diptera: Conopidae) of British Columbia, the Yukon, and Alaska

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# **ABSTRACT**

The thick-headed flies (Diptera: Conopidae) are rarely observed parasitoids. Confirmed hosts include many species of bees and wasps. Often collected from flowers, conopids may serve as either pollinators or pollinator predators. The last detailed checklist of the Conopidae of British Columbia was published in 1959. An updated checklist for British Columbia, the Yukon, and Alaska is presented based on over 1,000 specimens and specimen records. Geographical distribution, using an ecoprovince approach, is documented for each of 26 species in the region. Host, plant association, and hilltopping behavioural records based on past literature and new observations are also included. An identification key to all species recorded is included.

Key words: parasitoid, biogeography, plant associations, host associations, Nearctic

# **INTRODUCTION**

Conopidae (thick-headed flies) is a small, rarely collected family within the acalyptrate Diptera. Many species are noted for their mimicry of wasps and bees. Adult female conopids deposit eggs within living hosts using modified abdominal structures, often in midflight. The larvae develop within the host, slowly consuming tissue, until the host succumbs. Pupation occurs inside the host. Adult eclosion from the host's corpse usually follows an overwintering period. Various species of Hymenoptera are reported as hosts, but confirmation of host status by rearing is rare (Gibson et al., 2014). The possible impact of Conopidae on pollinator communities has been the focus of some research (e.g., Schmid-Hempel and Schmid-Hempel 1996, Gillespie 2010, Malfi and Roulston 2013). Conopids are also regularly collected from flowers but their role as pollinators, or even their degree of plant specificity, is poorly documented. Studies investigating specific flower associations or possible roles as pollinators have been few and limited (Freeman 1966, Maeta and Macfarlane 1993).

Other aspects of Conopidae life history are understudied. Some species engage in hilltopping behaviour (Mei et al. 2010), where males gather on hilltops or other prominent geographical features to await females. The degree to which hilltopping strategies are used by different species of Conopidae is poorly known.

Worldwide, more than 800 species of Conopidae – organized into six subfamilies and 59 genera and subgenera – are currently described (Gibson and Skevington 2013). Species live in every region and continent except Antarctica and the Pacific Islands. Williston (1882, 1883, 1885) described a large number of the western Nearctic species and summarized the current knowledge in a series of papers. Later studies of Nearctic species include Van Duzee's (1927) review of California Academy of Sciences (CAS) specimens and Parsons' (1948) analysis of material from Harvard's Museum of Comparative Zoology (MCZ). The tireless work of Sid Camras includes revisions of

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individual North American genera (Camras 1943, 1944, 1953, 1955, 1957), regional analyses (Camras and Hurd 1957), and continental catalogues (Camras 1965).

The Canadian summary of insect diversity (McAlpine et al. 1978) lists 30 species of Conopidae, with fifteen more likely to be discovered or described. The most recent review of the Conopidae fauna of British Columbia was that of Smith (1959), whose checklist, based on 104 specimens from the Spencer Entomological Museum (University of British Columbia) collection, included eighteen species in six genera. Smith did not draw any conclusions about the intraprovincial distribution of each species. Neither *Insects of the Yukon* (Danks 1997), nor *Arctic Arthropods* (Danks 1981), mentions Conopidae. An updated list of conopid species in the northwestern Nearctic, along with a summary of all known host and plant associations is necessary to assess the true biodiversity and ecological impact of this family in the region.

# **MATERIALS AND METHODS**

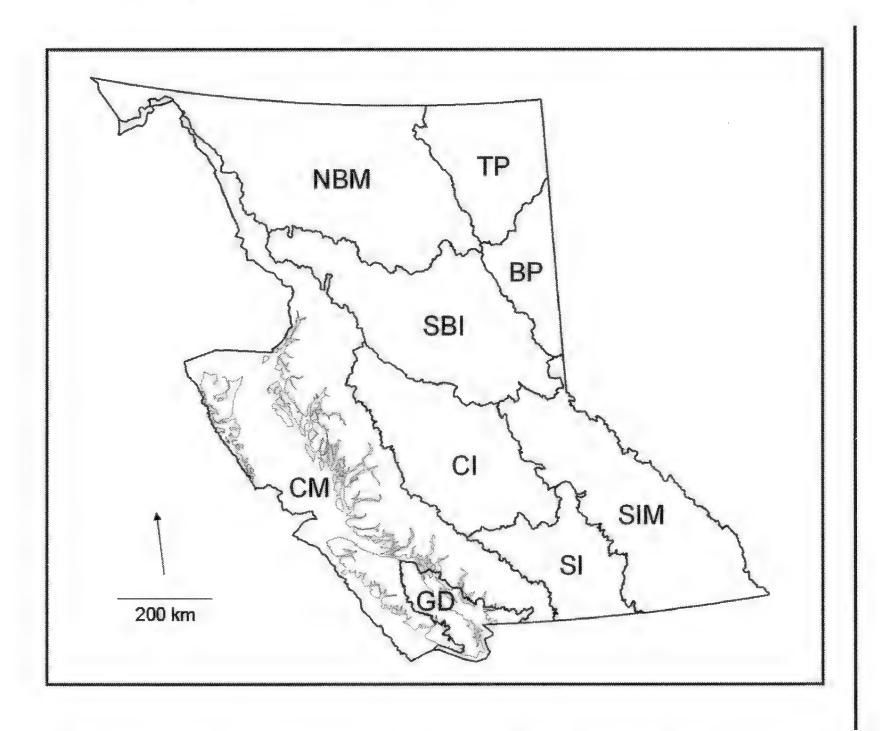
Taxonomic classification follows that of Gibson and Skevington (2013). Morphological features are as described in Gibson and Skevington (2013) and Gibson et al. (2013). Previous records of Conopidae confirmed from British Columbia, Alaska, and the Yukon are tallied. Records include specimens loaned to the author as well as digital databases of specimens with confirmed identifications. All notes regarding collection date, plant associations, rearing from hosts, or hilltop locations are recorded. Previous literature was examined to gather data on species range, host records, and plant associations. Specimens examined, or confirmed specimen records, are listed in each species account and are housed in the following collections: University of Calgary Museum of Zoology, Calgary, Alberta (BDUC); California Academy of Sciences, San Francisco, California (CAS); Canadian National Collection of Insects, Arachnids, and Nematodes, Ottawa, Ontario (CNC); University of Guelph Insect Collection, Guelph, Ontario (DEBU); Essig Museum of Entomology, University of California, Berkeley, California (EMEC); Royal BC Museum, Victoria, British Columbia (RBCM); Royal Ontario Museum Entomology Collection, Toronto, Ontario (ROME); Royal Saskatchewan Museum, Regina, Saskatchewan (RSM); Spencer Entomological Collection (Beaty Biodiversity Museum), University of British Columbia, Vancouver, British Columbia (SEM); University of Alaska Museum Entomology Collection, Fairbanks, Alaska (UAM); National Museum of Natural History, Washington, D.C. (USNM); William F. Barr Entomological Collection, University of Idaho, Moscow, Idaho (WFBM); Wallis-Roughley Museum of Entomology, University of Manitoba, Winnipeg, Manitoba (WRME); James Entomological Collection, Washington State University, Pullman, Washington (WSU).

An ecoprovince approach, similar to that of Ratzlaff (2015), is employed. Ecoprovinces are defined as those in Demarchi (2011), Smith et al. (2004), and Gallant et al. (1995). Ecoprovinces have been used in other recent insect checklists for British Columbia (Scudder and Cannings 2009, Ratzlaff 2015) to summarize insect distributions. With this method, the province is divided into area based on climatic, topographic, and geological similarity (Demarchi 2011). There are ten ecoprovinces in BC (Fig. 1), each of which could be considered unique sets of habitats. The presence of each conopid species within these ecoprovinces has been recorded.

# **RESULTS AND DISCUSSION**

Analysis of 1,016 specimens and specimen records has produced a list of 26 species of Conopidae in British Columbia (Table 1). Six of these species also occur in Yukon, and three of them are known in Alaska. This represents nine species added to Smith's (1959) checklist and, for sixteen more species, geographical ranges in BC, Yukon, and Alaska are expanded. A complete list of all specimens included in the analysis –

including collection localities, collectors, dates, and repositories – has been uploaded to figshare (DOI: 10.6084/m9.figshare.5373361).



**Figure 1.** British Columbia ecoprovinces as adapted from Scudder and Cannings (2009). Ecoprovince abbreviations: GD – Georgia Depression; CM – Coast and Mountains; SI – Southern Interior; SIM – Southern Interior Mountains; CI – Central Interior; SBI – Southern Boreal Interior; NBM – Northern Boreal Mountains; BP – Boreal Plains; TP – Taiga Plains.

Say (1823) describes *Physocephala marginata* from Missouri. Parsons (1948) reports the range of this species as Kansas and Texas and East to Massachusetts, with isolated individual specimens from Quebec, Ontario, Wyoming, and the Nicola Valley, British Columbia. The inclusion of British Columbia in the range of *P. marginata* in this paper and inclusion in Smith's (1959) subsequent list is likely based on a single misidentified specimen. Analyses recovered no specimens from British Columbia, Yukon, Alberta, Saskatchewan, or Manitoba. This species likely does not occur in Canada west of Ontario. (Note – this species has not been included in the summary of species in Table 1). Confirmed rearing records from other regions were found for nine species. Plant associations were recorded for all but one species. Hilltopping behaviour, including observations of hilltopping in BC, was recorded for five species. Detailed discussion of patterns of distribution and ecological associations follows a complete species checklist.

## Key to species of Conopidae found in British Columbia, Yukon, and Alaska

- Labella, shorter than prementum, broad, separate for entire length, projecting forward from apex of prementum; shiny patch present near apex of metatibia;

	vein CuA <sub>2</sub> curved along its length; female abdominal tergite and sternite 5
	fused; male cerci attached by narrow, sclerotized stalk
2.	Ocellar and postocellar bristles absent; basisternum broad; veins Sc and R <sub>1</sub>
	separate for entire length; female abdominal tergite and sternite 6 completely
	separate; female abdominal segment 7 laterally compressed along entire length;
	phallus visible, elongate, ribbon-shaped, setose along entire length
-	Ocellar and postocellar bristles present; basisternum short, narrow, single
	sclerite; veins Sc and R <sub>1</sub> fused before reaching costa; female abdominal tergite
	and sternite 6 at least partly fused; female abdominal segment 7 rounded at least
	in basal half; phallus usually not visible externally Myopinae 5
3.	Small species; total body length <4mm. Scutellum and femora entirely
	black
	Larger; total body length >6mm. Scutellum and femora with some yellow4
1	
4.	Dorsal hairs black. Wings smoky Dalmannia blaisdelli Cresson 1919
ene	Dorsal hairs white. Wings hyaline
5.	Stout, reddish flies with a large white head; gena at least one third of total head
	height; anterior margin of subcranial cavity rounded; costa uniform thickness
	along entire length Myopa 6
•••	Small (<10mm total length) black, shining flies; gena less than one third of head
	height; anterior margin of subcranial cavity straight; costa thickened at endpoint
	of $Sc+R_1$
6.	Wings with spots or with cross-veins clouded
	Wings completely hyaline
7.	Wings with only cross-veins clouded
1.	Wings with only cross-veins clouded
***	
0	
8.	Abdomen almost entirely black
	Abdomen almost entirely red; with or without pollinosity
9.	Abdomen dorsally with long, black hairs Myopa longipilis Banks 1916
9000	Abdomen dorsally with short, pale hairs Myopa vesiculosa Say 1823
10.	Abdomen with dense, long black hairs dorsally; (this species is easily confused
	with M. clausa and M. rubida) Myopa curticornis Kröber 1916
	Abdomen with sparse, short black hairs dorsally
11.	Abdomen dorsally with extensive pollinosity; (this species is easily confused
	with M. curticornis and M. rubida)
-	Abdomen dorsally without extensive pollinosity; (this species is easily confused
	with <i>M. clausa</i> and <i>M. curticornis</i> )
12.	Hind femora entirely black; abdomen with entirely pale hairs
1 4.	
1.0	Hind femora at least partly yellow; abdomen with at least some black hairs 13
13.	Large species (total body length >6mm); hind femora almost entirely yellow
	Thecophora modesta (Williston 1883)
-	Smaller species (total body length <6mm); hind femora partly yellow and partly
	black
14.	Less than one third of hind femora yellow; (this species is easily confused with
	T. nigripes and T. occidensis) Thecophora luteipes (Camras 1945)
_	One third to three quarters of hind femora yellow
15.	One third to one half of hind femora yellow; (this species is easily confused with
IJ.	T. luteipes and T. occidensis) Thecophora nigripes (Camras 1945)
	More than one half of hind femora yellow (this species is easily confused with T.
	· · · · · · · · · · · · · · · · · · ·
	luteipes and T. nigripes) Thecophora occidensis (Walker 1849)

16.	First abdominal segment as broad as thorax; arista mid-dorsal; second aristomere equal in length to first; scape quadrate; ocellar tubercle well-developed; ocellar and postocellar bristles present; anterior margin of subcranial cavity rounded; maxillary palpal length at least equal to prementum width; basisternum narrow, divided posteriorly, with elongate and narrow posterolateral extensions; more than two pairs of scutellar bristles usually present; vena spuria absent; epandrium separate beyond cerci
-	First abdominal segment narrow and thread-like; arista stylate and apical; second aristomere usually expanded ventrally; scape at least twice as long as wide; ocellar tubercle reduced or absent; ocellar and postocellar bristles absent; anterior margin of subcranial cavity projecting forward at junction with medial carina; maxillary palpi reduced or absent; basisternum broad, posterolateral extensions short and blunt; zero or one pair of scutellar bristles; vena spuria present; epandrium fused beyond cerci
17.	Thorax dark with white or golden stripes
18.	Thorax either entirely dark or lighter with darker spots or stripes
-	Larger species (total body length >5mm); thorax variable, but usually dark, sometimes with darker markings
19.	Smaller species (total body length <6mm); almost entirely grey with some darker markings
-	Larger species (total body length >6mm); grey to dark grey with some reddish
20.	Third tergite of female abdomen longer than all other tergites; (males may be
-	indistinguishable from Z. cinereiventre) Zodion perlongum Coquillet 1902 Third tergite of female abdomen equal in length or shorter than at least one other tergite; (males may be indistinguishable from Z. perlongum)
21.	Abdomen with extensive red colouration; (this species is easily confused with <i>Z. intermedium</i> )
-	Abdomen without red colouration or with red limited to outer margins; (this species is easily confused with <i>Z. fulvifrons</i> )
22.	Ocelli and ocellar tubercle absent; ventral half of proepisternum bare; prominent row of setae on posterior surface of mesofemur absent; metafemur expanded proximally
-	Three ocelli present; ocellar tubercle present; ventral half of prosepisternum with setae and/or bristles; prominent row of setae on posterior surface of mesofemur present; metafemur parallel-sided along entire length
23.	Colouration dark to black throughout, especially on frontal markings  Physocephala furcillata (Williston 1882)
90a	Colouration reddish throughout, especially on frontal markings
24.	Black markings on scutum limited to a single central stripe; gena uniformly dark
_	
	entire dorsal surface; gena with paler central spot (this character often difficult to see)
25.	Frons, second abdominal tergite, and all of the scutum very dark to black

Table 1
Species of Conopidae recorded in British Columbia, Yukon, and Alaska by Smith (1959) and the present study.

	British Columbia							Yukon	Alaska	
Species	GD	CM	SI	SIM	CI	SBI	NBM	BP		
Conopinae		***************************************		***************************************	44 x x x 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	*************		************************		************************************
Physocephala burgessi	S,G	S,G	S,G	G	S,G					
Physocephala furcillata *								G		
Physocephala texana			S,G	G	S,G					
Physoconops fronto *			G							
Physoconops obscuripennis			S,G	G						
Dalmanniinae										
Dalmannia blaisdelli			S,G							
Dalmannia picta *			G	G						
Dalmannia vitiosa *				G						
Myopinae										
Myopa clausa	G		S,G	G	S,G					
Myopa curticornis *	G		G	G					G	G
Myopa longipilis	S,G		G	G						
Myopa rubida	S,G		S,G	G					G	
Myopa vesiculosa			S,G	G	S				G	
Myopa vicaria	S,G		$\mathbf{G}$	G	$\mathbf{G}$		G	S,G	G	G
Myopa willistoni *	G		$\mathbf{G}$							
Thecophora luteipes	S,G		G	G	G					
Thecophora modesta	S,G	G	S,G	G	S					
Thecophora nigripes	G		G		S,G	G				
Thecophora occidensis	G	G	G	G	S,G				G	G
Thecophora propingua	S,G		S,G	G	S					
Zodioninae										
Zodion americanum *			G	G						
Zodion cinereiventre *			G	G						
Zodion fulvifrons	G	G	$\mathbf{G}$	G	S,G					
Zodion intermedium		G	G	G	S,G		G	G		
Zodion obliquefasciatum *			G							
Zodion perlongum	G		G		S					

<sup>\* -</sup> species recorded in British Columbia for the first time. S – species recorded in each region by Smith 1959. G – Species recorded in each region by the present study. Ecoprovince abbreviations: GD – Georgia Depression; CM – Coast and Mountains; SI – Southern Interior; SIM – Southern Interior Mountains; CI – Central Interior; SBI – Southern Boreal Interior; NBM – Northern Boreal Mountains; BP – Boreal Plains.

# **Species Checklist**

CONOPINAE

Physocephala burgessi (Williston 1882)

Specimens or records observed: CAS, CNC, DEBU, EMEC, RBCM, RSM, SEM, WRME, WSU. BC: Alta Lake, Apex Mountain, Bamberton Provincial Park, Clinton, Cobble Hill, Courtenay, Cranbrook, Crowsnest Pass, Errington, Fitzgerald, Flathead, Fort Langley, Forward Harbour, Gang Ranch Junction, Goldstream, Hope, Jesmond, Kaslo, Keremeos, Kishinena Creek, Kleena Kleene, Maple Bay, Mount Alava, Mount Cain, Mount Kobau, Mount Seymour, Nanaimo, Nelson, Ocean Falls, Osoyoos, Pemberton, Qualicum, Quesnel, Revelstoke, Robson, Saanich, Salmon Arm, Salvus, Savary Island, Sayward, Seton Lake, Shawnigan, Sidney, Squamish, Stagleap Provincial Park,

Strathcona Provincial Park, Terrace, Tulameen, Upper Carmanah Valley, Vancouver, Vaseux Lake, Vernon, Victoria, Walhachin, Wellington, Whistler.

Distributional notes: Williston's (1882) description is based on type specimens from Colorado and California. Parsons (1948) records the range as Montana to New Mexico and west to California. Camras and Hurd (1957) and Camras (1957, 1965) list the range for this species as Alberta to Texas and West to the Pacific Ocean. Smith (1959) includes this species in his list for British Columbia. Analyses of other specimens indicate that within Canada, *P. burgessi* has only been detected in British Columbia and Alberta.

Flight period: June - August

Ecological associations: In California, *P. burgessi* has been collected from *Prunus* sp. (Rosaceae) and *Ceanothus* sp. (Rhamnaceae) (Bohart 1941). Camras and Hurd (1957) report *Bombus sonorus* Say 1837 (Apidae) as a host. Males were collected from the summits of Mount Kobau, Mount Cain, and Mount Finlayson.

Physocephala furcillata (Williston 1882)

Specimens or records observed: CNC, RBCM. BC: Chetwynd, Fort St. John, Hudson's Hope, Rolla.

Distributional notes: Williston (1882) describes the species from New Hampshire. Parsons (1948) records it from Wisconsin to Atlantic Canada, south to New Jersey, but also in Mexico and California. Camras and Hurd (1957) and Camras (1957, 1965) report this species as found from Atlantic Canada, south to Pennsylvania and West to Alberta, but also possibly in California and Mexico. Analyses of other specimens indicate that *P. furcillata* is present in every Canadian province except Newfoundland and Labrador.

Flight period: June

Ecological associations: One specimen observed from Manitoba was reared from *Bombus terricola* Kirby 1837. MacFarlane and Pengelly (1975) reared this species from *Bombus vagans* Smith 1854 in Ontario. Specimens were collected from *Solidago* sp. (Asteraceae), *Arctium* sp. (Asteraceae), and *Chamerion angustifolium* (Onagraceae) flowers. Mei et al. (2010) suggested that this species is a likely hilltopper based on specimens collected in the Ottawa area.

Physocephala texana (Williston 1882)

Specimens or records observed: CNC, DEBU, RBCM, ROME, RSM, WRME. BC: Ashcroft, Cascade, Castlegar, Chilcotin, Chopaka, Christina Lake, Clinton, Cranbrook, Dog Lake, Edgewood, Fairview, Farwell Canyon, Flathead Valley, Gang Ranch Junction, Inkaneep Provincial Park, Kamloops, Keremeos, Lillooet, Midway, Mount Kobau, Nicola River, Oliver, Osoyoos, Penticton, Robson, Soda Creek, Summerland, Vaseux Creek, Vaseux Lake, Vernon, Walhachin.

Distributional notes: Williston (1882) describes the species based on specimens from California, Texas, and Kansas. Parsons (1948) documents the range of *P. texana* as California to Georgia, with an additional specimen from Quebec. Camras and Hurd (1957) list it throughout the USA, but rare in the west; and Camras (1957, 1965) reports it occurring throughout the USA, Canada, and into Mexico. Smith (1959) includes *P. texana* in his list for British Columbia and other data indicate that it ranges east to Quebec and Nova Scotia.

Flight period: June - September

Ecological associations: This is one of the few species confirmed as a parasitoid of honey bees (*Apis mellifera* Linnaeus 1758 (Apidae)). It has been reared from commercial bees in Wyoming and Washington (Van Duzee 1934, Riedel and Shimanuki 1966). In California, it has been seen to attack, oviposit in, and emerge from *Bembix occidentalis buettenmuelleri* Fox 1901 (Crabronidae) and *B. comata* Parker 1917(Bohart and MacSwain 1939, 1940). It was reared from *Nomia melanderi* Cockerell 1906 (Halictidae) in Idaho (Foote and Gittins 1961). Hobbs (1965, 1966) also reported this species as "killing" queens of *Bombus rufocinctus* Cresson 1863 and *B. fervidus* (Fabricius 1798) in

southern Alberta. It was also reared from *B. bifarius* Cresson 1878, *B. californicus* Smith 1854, *B. flavifrons* Cresson 1863, and *B. occidentalis* Greene 1858 in Alberta (Otterstatter et al. 2002).In California, *P. texana* frequents and mates on flowers of *Eriogonum* sp. (Polygonaceae) and *Heliotropium* sp. (Boraginaceae) (Bohart and MacSwain 1939). Freeman (1966) reports an association between this species and flowers of *Asclepias fascicularis* (Apocynaceae), *Achillea millefolium* (Asteraceae), *Melilotus alba* (Fabaceae), *Mentha* sp. (Lamiaceae), and *Chrysothamnus* sp. (Asteraceae). It hilltops in Quebec (Mei et al. 2010).

Physoconops (Physoconops) fronto (Williston 1885)

Specimens or records observed: CAS. BC: Vernon.

Distributional notes: Williston (1885) describes this species from Kansas. Parsons (1948) lists the range as Nebraska to Texas, west to California, with a single specimen from Massachusetts. Camras and Hurd (1957) and Camras (1955, 1965) describe the range as Massachusetts to Florida, west to California and Washington, south to Mexico. Other specimens indicate that *P. fronto* occurs in British Columbia, Alberta, and Manitoba.

Flight period: August

Ecological associations: Bohart and MacSwain (1940) reared a specimen of *Conops argentifacies* VanDuzee (a synonym of *P. fronto*) from *Megachile (Xanthosaurus) perihirta* Cockerell 1898 (Megachilidae). Foote and Gittins (1961) reared it from a nesting site of *Nomia melanderi*. Freeman (1966) lists the following plant associations for *P. fronto*: *Asclepias fascicularis*, *Chrysothamnus* sp., *Daucus carota* (Apiaceae), *Melilotus alba*, and *Solidago* sp.

Physoconops (Physoconops) obscuripennis (Williston 1882)

Specimens or records observed: CNC, RBCM, SEM. BC: Kamloops, Oliver, Osoyoos, Penticton, Robson.

Distributional notes: Williston (1882) describe this species from South Carolina. Parsons (1948), Camras and Hurd (1957), and Camras (1955, 1965) give its range as Massachusetts to Florida, west to Alberta, British Columbia and Washington, likely in California. Smith (1959) includes this species in his list for British Columbia. Other Canadian specimens of *P. obscuripennis* are from British Columbia, Alberta, Manitoba, and Ontario.

Flight period: June - July

Ecological associations: Freeman (1966) reports this species on flowers of *Cirsium arvense* (Asteraceae), *Melilotus alba*, and *Solidago* sp.

#### **DALMANNIINAE**

Dalmannia blaisdelli Cresson 1919

Specimens or records observed: CNC, RBCM, SEM. BC: Kilpoola Lake, Old Hedley Road, Oliver, Penticton, Vaseux Creek, Vernon.

Distributional notes: The original description by Cresson (1919) lists Colorado as the type locality with paratypes from California. Bohart (1938) only reported specimens from California. Camras and Hurd (1957) and Camras (1965) list the range as Colorado and Wyoming, west to Oregon and California. Analyses of other Canadian specimens indicate that *D. blaisdelli* has only been detected in British Columbia.

Flight period: May

Ecological associations: Bohart (1938) mentions that the species is associated with heavily wooded areas.

Dalmannia picta Williston 1883

Specimens or records observed: CNC, SEM. BC: Oliver, Robson.

Distributional notes: In the original description, Williston (1883) lists the type locality as New Mexico. Bohart (1938) and Parsons (1948) record specimens from Arizona and California. Camras and Hurd (1957) and Camras (1965) list the range for this species as British Columbia to New Mexico, west to California. Smith (1959) includes this species in his list for British Columbia. Analyses of other specimens indicate that it occurs nowhere else in Canada.

Flight period: May - June

Ecological associations: Bohart (1938) notes that specimens in the Mojave Desert, California were collected near large aggregations of *Diandrena* sp. (Andrenidae) bees. Freeman (1966) mentions *Brassica nigra* (Brassicaceae) as a plant association.

# Dalmannia vitiosa Coquillet 1892

Specimens or records observed: CNC. BC: Robson.

Distributional notes: Coquillet (1892) describes the species based on a specimen from Los Angeles, California. Bohart (1938) gives it a wide range (California, Virginia, Kansas) and Parsons (1948) lists specimens from New Hampshire to Virginia, plus California, Kansas, Arizona, and Nevada. Camras and Hurd (1957) and Camras (1965) list the distribution as patchy across North America from Atlantic to Pacific. Analyses of other specimens indicate that *D. vitiosa* has been collected in all Canadian provinces except Manitoba, Prince Edward Island, and Newfoundland and Labrador.

Flight period: May - June

Ecological associations: Specimens were observed on *Cornus* sp. (Cornaceae) blossoms in Alberta. This species might demonstrate hilltopping behaviour based on observations from Ontario and Quebec (Mei et al. 2010).

#### MYOPINAE

Myopa clausa Loew 1866

Specimens or records observed: CNC, RBCM, SEM. BC: Agassiz, Aspen Grove, Bowser, Chilcotin, Courtenay, Creston, Kamloops, Kelowna, Keremeos, Oliver, Penticton, Quesnel, Robson, Saanich, Sorenson Lake, Summerland, Vancouver, Victoria, Yale.

Distributional notes: Loew's (1866) type specimen is from Maine. Williston (1885) lists the range as New England. Banks (1916) records it only in the East. Parsons (1948), as well, limits the range from Maine to North Carolina, and possibly from Iowa, Arizona, Washington, Wyoming, and California. However, Camras and Hurd (1957) and Camras (1953, 1965) give the distribution of *M. clausa* as Maine to Georgia, west to British Columbia and California. Smith (1959) includes it in his list for British Columbia. Analyses of other specimens indicate that *M. clausa* occurs in all Canadian provinces except Manitoba, Prince Edward Island, and Newfoundland and Labrador.

Flight period: April - June

Ecological associations: Specimens from Quebec have been collected from *Viburnum acerifolium* (Adoxaceae) flowers. Mei et al. (2010) concluded that this species may be an exclusive hilltopper based on specimens observed in the Ottawa region.

Myopa curticornis Kröber 1916

Specimens or records observed: RBCM, SEM, UAM. AK: Fairbanks; BC: Cranbrook, Hatzic, Penticton, Robson, Salmon Arm, Vancouver, Vaseux Lake, Vernon, Wellington; YT: Ross River.

Distributional notes: Kröber's (1916) type specimens are from Colorado and California. Parsons (1948) mentions specimens from Washington, Oregon, California, Utah, Colorado, and Maine. Camras and Hurd (1957) and Camras (1953, 1965) list the range as Wyoming to Arizona, west to Washington and California. Analyses of other specimens indicate that, in Canada, *M. curticornis* only lives in British Columbia and Yukon.

Flight period: April - June

Ecological associations: Specimens from Alaska have been collected from *Prunus padus* and *Salix alaxensis* (Salicaceae) flowers.

Myopa longipilis Banks 1916

Specimens or records observed: CNC, RBCM, SEM. BC: Agassiz, Kamloops, Oliver, Osoyoos, Penticton, Robson, Vancouver, Vernon.

Distributional notes: Banks' (1916) original types are from Washington State. Parsons (1948) mentions specimens from Oregon and California. Camras and Hurd (1957) and Camras (1953, 1965) list the range as British Columbia to Utah, west to California. Smith (1959) includes this species in his list for British Columbia. Analyses of other specimens indicate that *M. longipilis* is known in Canada from only British Columbia and Alberta.

Flight period: April - May

Ecological associations: Freeman (1966) reports this species from Prunus subcordata.

Myopa rubida (Bigot 1887)

Specimens or records observed: CAS, RBCM, SEM. BC: Highlands, Robson, Saanich, Vernon, Victoria; YT: Stewart Crossing.

Distributional notes: Bigot's (1887) types are from Colorado. Banks (1916) records the species from Oregon and Washington. Camras and Hurd (1957) and Camras (1953, 1965) list the range as west of the Rocky Mountains. Smith (1959) includes this species in his list for British Columbia. In Canada, *M. rubida* occurs in British Columbia, Alberta, and Yukon.

Flight period: May - July

Ecological associations: MacSwain and Bohart (1947) successfully reared this species from *Andrena vierecki* Cockerell 1904 (Andrenidae) in California. Smith (1959) reports it from *Capsella bursa-pastoris* (Brassicaceae). Freeman (1966) lists additional plants visited by this species: *Brassica campestris*, *Prunus* sp., and *Ranunculus californicus* (Ranunculaceae). A male was collected from the summit of Lone Tree Hill (Highlands, BC).

Myopa vesiculosa Say 1823

Specimens or records observed: CAS, RBCM, SEM, CNC. BC: Cranbrook, Grand Forks, Grindrod, Kamloops, Osoyoos, Penticton, Robson, Salmon Arm, Vernon; YT: Ross River, Stewart Crossing.

Distributional notes: Say (1823) describes the species based on specimens from Pennsylvania. Williston (1885) lists the species only in the eastern United States. Banks (1916) records a specimen from Nebraska. Parsons (1948) mentions specimens from New Hampshire to Virginia, west to Washington, while Camras and Hurd (1957) and Camras (1953, 1965) list the range for this species as Quebec to Florida, west to Washington and California. Smith (1959) includes *M. vesiculosa* in his list for British Columbia and analyses of other specimens indicate that it lives in the Yukon and all Canadian provinces except Prince Edward Island and Newfoundland and Labrador.

Flight period: April - June

Ecological associations: Specimens from British Columbia were collected from flowers of *Sorbus* sp. (Rosaceae). This species may be an occasional hilltopper in Ontario and Quebec (Mei et al. 2010).

Myopa vicaria Walker 1849

Specimens or records observed: CAS, CNC, RBCM, SEM, UAM. AK: Fairbanks; BC: Atlin, Chilcotin, Cranbrook, Kamloops, Lavington, Nelson, Oliver, Peace River, Penticton, Robson, Vancouver, Vernon; YT: Rampart House.

Distributional notes: The type for this species, described by Walker (1849), is from Nova Scotia. Parsons (1948) gives the range as Nova Scotia to Virginia, west to Illinois, plus specimens from Washington, Oregon, Wyoming, and Arizona, while Camras and Hurd (1957) and Camras (1953, 1965) list it as Nova Scotia to Georgia, west to Alaska and California. Smith (1959) includes this species in his list for British Columbia; it occurs in the Yukon and all Canadian provinces except Prince Edward Island and Newfoundland and Labrador.

Flight period: April - May

Ecological associations: This species has been collected from various species of willow (*Salix alaxensis*, *S. arbusculoides*, *S. planifolia*, *S. pulchra*, *S. scouleriana*) in Alaska and also *Salix* sp. in Alberta. In their study in the Ottawa region, Mei et al. (2010) did not find this species on hilltops.

## Myopa willistoni Banks 1916

Specimens or records observed: CNC, RBCM, SEM. BC: Caulfield, Summerland, Vancouver, Vaseux Lake, Vernon.

Distributional notes: Williston (1885) originally describes this species as *M. pictipennis*, which is a preoccupied name, from Arizona and California; Banks (1916) provided the new species name and saw specimens from Oregon and California. Camras and Hurd (1957) and Camras (1953, 1965) list the range as west of the Rocky Mountains, south into Mexico. Analyses of other specimens indicate that *M. willistoni* has only been found in Canada in British Columbia.

Flight period: May

Ecological associations: None noted.

# Thecophora luteipes (Camras 1945)

Specimens or records observed: CAS, DEBU, RBCM, SEM. BC: Hell's Gate, Penticton, Robson, Sparwood, Thetis Lake, Vernon, Westwick Lake.

Distributional notes: Camras (1945) describes this species based on specimens from Colorado, Washington, Idaho, Utah, and California. Camras and Hurd (1957) and Camras (1965) record the range as British Columbia to Colorado, west to California. Smith (1959) includes this species in his list for British Columbia and examination of other specimens shows that, in Canada, it only occurs in that province.

Flight period: June - September

Ecological associations: Freeman (1966) summarizes plant associations for this species as: *Crepis virens* (Asteraceae), *Daucus carota*, *Eriogonum elatum*, and *Trifolium repens* (Fabaceae).

#### Thecophora modesta (Williston 1883)

Specimens or records observed: CAS, CNC, RBCM, SEM. BC: Agassiz, Chase, Clearwater, Comox, Creston, Hope Mountains, Kootenay Lake, Lillooet, Metchosin, Midday Creek, Mount Kobau, Newgate, Okanagan, Oliver, Robson, Salmo, Vancouver, Vernon, Victoria, Walhachin; YT: Dawson.

Distributional notes: Williston (1883) describes this species based on specimens from California and Washington. Camras and Hurd (1957) and Camras (1945, 1965) record the range of this species as Saskatchewan to New Mexico, west to the Pacific Ocean. Smith (1959) includes this species in his list for British Columbia; it also occurs in Yukon and Alberta.

Flight period: June - September

Ecological associations: Cole and Lovett (1921) report *Halictus ligatus* Say 1837 (Halictidae) as a host for this species in Oregon. Freeman (1966) includes: *Anaphalis* sp. (Asteraceae), *Brassica rapa*, *Cirsium* sp., *Solidago* sp., and *Trifolium hybridum* as plant associations. Individuals have been observed on the summits of Mount Tolmie and Camas Hill (BC: Victoria region).

#### Thecophora nigripes (Camras 1945)

Specimens or records observed: CNC, RBCM, SEM. BC: Australian, Burton, Clinton, Merritt, Mount Kobau, Oliver, Penticton, Prince George, Saanich, Vancouver, Westwick Lake.

Distributional notes: Camras (1945) describes this species based on a specimen from Thunder Bay, Ontario, but mentions 132 paratypes from across Canada, USA, and Guatemala. All subsequent works (Parsons 1948, Camras and Hurd 1957, Camras 1965) record the range as Nova Scotia to Georgia, west to British Columbia and California, south to Guatemala. Smith (1959) includes this species in his list for British Columbia. *T. nigripes* has been found in all Canadian provinces except New Brunswick, Prince Edward Island, and Newfoundland and Labrador.

Flight period: July - August

Ecological associations: Plant associations for this species (Freeman 1966) include *Chrysothamnus* sp., *Crepis virens*, *Chamerion augustifolium* (Onagraceae), *Prunus* sp., and *Solidago* sp. Mei et al. (2010) are unsure if the species hilltops in the Ottawa area.

# Thecophora occidensis (Walker 1849)

Specimens or records observed: BDUC, CAS, CNC, RBCM, SEM, UAM. AK: Fairbanks, Matanuska; BC: Agassiz, Burton, Chilcotin, Cowichan, Cottonwood River, Crowsnest, Flathead Valley, Galiano Island, Hedley, Hope Mountains, Kalamalka Lake, Kootenay, Langford, Lytton, Mahoney Lake, Mount Kobau, Nanaimo, Nicola, Oliver, Osoyoos, Penticton, Quesnel, Robson, Salmo, Sheep Lake, Soda Creek, Sparwood, Strathcona Provincial Park, Thetis Lake, Vancouver, Vernon, Victoria, Walhachin, Westwick Lake; YT: Carmacks, Dawson, Lone Tree Creek, Old Crow, Starr Creek, Tagish, Whitehorse.

Distributional notes: Walker (1849) describes the species based on a specimen from Ohio. Camras (1945), Parsons (1948), and Camras and Hurd (1957) describe the range of *Occemyia loraria*, a synonym of *T. occidensis*, as throughout the USA and southern Canada. Camras (1965) records the range of *T. occidensis* as Quebec to Georgia, west to the Yukon and California, south to Mexico. Smith (1959) includes *O. loraria* Loew 1866 in his list for British Columbia. It also lives in Yukon, Northwest Territories, and all Canadian provinces except New Brunswick and Prince Edward Island.

Flight period: June - September

Ecological associations: This species has been reared from *Halictus confusus* Smith 1853, *H. ligatus*, *H. rubicundus* (Christ 1791), *Lasioglossum cinctipes* (Provencher 1888), *L. forbesii* (Robertson 1890), *L. imitatum* (Smith 1853), *L. laevissimum* (Smith 1853), and *L. lineatulum* (Crawford 1906) in Ontario (Smith 1966, Knerer and Atwood 1967). In his list, Freeman (1966) lists plant associations for *T. loraria* as: *Brassica campestris*, *Chrysothamnus* sp., *Daucus carota*, *Hypericum perforatum* (Hyperiaceae), *Melilotus* sp., and *Solidago* sp. This species may or may not demonstrate hilltopping behaviour in Ontario and Quebec (Mei et al. 2010).

## Thecophora propingua (Adams 1903)

Specimens or records observed: CAS, CNC, RBCM, SEM. BC: Cranbrook, Erickson, Kamloops, Lytton, Midway, Mount Kobau, Osoyoos, Penticton, Robson, Saanich, Saturna Island, Vernon.

Distributional notes: Adams (1903) does not provide a locality for the type. Parsons (1948), Camras and Hurd (1957), and Camras (1965) record the range as Nova Scotia to Alabama, west to British Columbia and California. Smith (1959) includes this species in his list for British Columbia. Analyses of other specimens indicate that *T. propinqua* occurs in all provinces from British Columbia to Quebec.

Flight period: May - September

Ecological associations: Specimens were collected on *Mentha* sp. from Vernon, British Columbia. Freeman's (1966) plant association list for this species includes: *Achillea millefolium*, *Amaranthus* sp. (Amaranthaceae), *Asclepias fascicularis*, *Brassica nigra*, *Chrysothamnus* sp., *Cleome lutea* (Cleomaceae), *C. serrulata*, *Daucus carota*, *D. pusillus*, *Eriogonum elatum*, *Grindelia* sp. (Asteraceae), *Medicago sativa* (Fabaceae), *Melilotus alba*, *Phacelia* sp. (Boraginaceae), *Solidago* sp., *Solanum tuberosum* (Solanaceae), *Triticum aestivum* (Poaceae). Mei et al. (2010) does not record any hilltopping in the Ottawa area.

#### ZODIONINAE

Zodion americanum Wiedemann 1830

Specimens or records observed: CNC, RBCM, SEM. BC: Burton, Creston, Dog Creek, Mount Kobau, Robson, Salmon Arm.

Distributional notes: Wiedemann's (1830) type specimen is from Uruguay. Camras, (1944, 1965), Parsons (1948), and Camras and Hurd (1957) list the range for this species as throughout Canada, USA, central America, and into South America and the Caribbean Islands. *Zodion americanum* has been recorded in all provinces except New Brunswick and Newfoundland and Labrador.

Flight period: June - September

Ecological associations: Freeman (1966) reports possible plant associates as *Melilotus alba* and *Solidago* sp. Mei et al. (2010) does not give evidence for hilltopping behaviour for this species in the Ottawa area.

## Zodion cinereiventre Van Duzee 1927

Specimens or records observed: CAS, CNC, RBCM. BC: Fernie, Mahoney Lake, Nicola, Osoyoos, Pavilion Lake, Penticton.

Distributional notes: The type specimen of Van Duzee (1927) is from California. Parsons (1948), Camras (1944), and Camras and Hurd (1957) give the range as throughout the USA, west of Illinois. Camras (1965) lists the range as Atlantic Canada to North Carolina, west to British Columbia and California. Other specimens indicate that *Z. cinereiventre* lives in all provinces from British Columbia to Ontario.

Flight period: June - August

Ecological associations: Freeman (1966) reports that possible plant hosts for this species include *Helenium tenuifolium* (Asteraceae) and *Senecio* sp. (Asteraceae).

Zodion fulvifrons Say 1823

Specimens or records observed: CNC, DEBU, RBCM, SEM, USNM, WFBC. BC: Bear Lake, Chilcotin, Cranbrook, Grand Forks, Hell's Gate, Jesmond, Junction Provincial Park, Kamloops, Kaslo, Kelowna, Lillooet, Lytton, Midway, Mount Kobau, Nelson, Nicola, Okanagan Falls, Oliver, Osoyoos, Penticton, Quesnel, Robson, Rock Creek, Royal Oak, Salmon Arm, Savary Island, Summerland, Vancouver, Victoria, Walhachin, Dog Creek.

Distributional notes: Say (1823) describes this species from Maryland and Pennsylvania. Camras (1944), Parsons (1948), and Camras and Hurd (1957) list the range as Atlantic Canada to Florida, west to Washington and California, south to Mexico. Smith (1959) includes this species in his list for British Columbia and other specimens show that *Z. fulvifrons* occurs in all Canadian provinces except Newfoundland and Labrador.

Flight period: May - August

Ecological associations: Severin (1937) reared this species from honey bees (Apis mellifera) from South Dakota. Foote and Gittins (1961) report it from flowers of Asclepias sp., Aster sp. (Asteraceae), Brassica sp., Chaenactis sp. (Asteraceae), Chrysothamnus sp., Eriogonum sp., and Trifolium repens in Idaho. In Alberta it has been

collected from *Solidago* sp. flowers. Mei et al. (2010) suggest that this species might hilltop in the Ottawa area.

#### Zodion intermedium Banks 1916

Specimens or records observed: CAS, CNC, DEBU, SEM, RBCM. BC: Boswell, Dog Creek, Enderby, Fort Steele, Hudson's Hope, Kamloops, Kinbasket Reservoir, Lillooet, Mount Kobau, Nicola, Oliver, Osoyoos, Penticton, Quesnel, Robson, Rock Creek, Sorrento, Telegraph Creek, Terrace, Vernon, White Lake.

Distributional notes: Banks (1916) describes this species from Pennsylvania. Camras (1944), Parsons (1948), and Camras and Hurd (1957) list the range as Atlantic Canada to Florida, west to Washington and California, south to Mexico. Smith (1959) includes this species in his list for British Columbia; *Z. intermedium* has been collected in all provinces except Newfoundland and Labrador.

Flight period: May - August

Ecological associations: Freeman (1966) summarizes plant associations for this species as: *Chrysothamnus* sp., *Brassica rapa*, *Erigeron canadensis* (Asteraceae), *E. linearis*, *Lupinus* sp. (Fabaceae), and *Solidago* sp. Specimens were observed from *Achillea* sp. in Alberta and a *Potentilla* (Rosaceae) meadow in British Columbia. Mei et al. (2010) suggests that this species might be a hilltopper in the Ottawa area.

# Zodion obliquefasciatum (Macquart 1846)

Specimens or records observed: CNC. BC: Penticton.

Distributional notes: Macquart's (1846) type specimen is from Texas. Parsons (1948), Camras (1965), and Camras and Hurd (1957) list the range as Wisconsin to Louisiana, west to Alberta, Washington, and California, south to Mexico. *Zodion obliquefasciatum* has been recorded from British Columbia to Manitoba.

Flight period: July - August

Ecological associations: Freeman (1966) summarizes plant associations for this species as: Chrysothamnus sp., Baileya pleniradiata (Asteraceae), Veronica sp. (Plantaginaceae), Centaurea repens (Asteraceae), Cirsium arvense, C. vulgare, Eriogonum sp., Gaillardia pulchella (Asteraceae), Grindelia sp., Gutierrezia microcephala (Asteraceae), Helianthus annuus (Asteraceae), H. petiolaris, Hemizonia fasciulata (Asteraceae), Heterotheca subaxillarias (Asteraceae), Medicago sativa, Melilotus alba, M. officinialis, Lupinus sp., Bahia absinthifolia (Asteraceae), Verbesina enceliodes (Asteraceae), Sphaeralcea angustifolia (Malvaceae), Asclepias sp., Solidago canadensis, and S. occidentalis.

# Zodion perlongum Coquillet 1902

Specimens or records observed: CNC. BC: Lillooet, Royal Oak.

Distributional notes: Coquillet (1902) describes this species from Colorado specimens. Camras (1944), Parsons (1948), and Camras and Hurd (1957) list the range as Maine to North Carolina, west to California, south to Mexico. Specimens examined indicate that *Z. perlongum* lives in British Columbia, Alberta, Saskatchewan, Ontario, Quebec, and Nova Scotia.

Flight period: June - August

Ecological associations: Freeman (1966) reports this species from flowers of *Chrysothamnus* sp.

Patterns of Distribution. Smith's (1959) checklist is limited in its data – only 104 specimens from a single collection (SEM) – and he drew no conclusions regarding provincial distributions of Conopidae. The present data, including many more records from more sources, allows some conclusions to be drawn. Nevertheless, most of the records and specimens examined are from a subset of locations within the region. In British Columbia, the Georgia Depression, Southern Interior, Central Interior, and

Southern Interior Mountains ecoprovinces are relatively well-collected (Table 1). Few specimens or records were noted from north of 53°N or from the coastal regions, including the western coast of Vancouver Island, the Gulf Islands, or Haida Gwaii. In Yukon, specimens or records from both the Boreal Cordillera and Taiga Cordillera are reported, but not in any other ecoprovinces. Specimens from Alaska are limited to the Cook Inlet and Interior Bottomlands ecoprovinces.

Based on recorded conopid distributions in British Columbia, Yukon, and Alaska, a few general geographical distribution patterns are evident. Some species can be best described as widespread, occurring in many regions of the northwestern Nearctic as well as across the continent. Such species include: Physocephala texana, Dalmannia vitiosa, Myopa clausa, M. vesiculosa, M. vicaria, Thecophora nigripes, T. occidensis, T. propinqua, Zodion americanum, Z. cinereiventre, Z. fulvifrons, Z. intermedium, and Z. perlongum. Other species appear to be limited to west of the Rocky Mountains: Physocephala burgessi, Dalmannia blaisdelli, D. picta, Myopa curticornis, M. longipilis, M. rubida, M. willistoni, Thecophora luteipes, and T. modesta. A few species are southern in distribution with only a limited incursion into British Columbia, mostly in warm Southern Interior valleys: Physoconops fronto, P. obscuripennis, Zodion obliquefasciatum. Physocephala furcillata occurs throughout Canada, but, in British Columbia, only east of the Rockies in the Peace region. Conopid species apparently able to tolerate conditions north of 60°N are Myopa curticornis, M. rubida, M. vesiculosa, M. vicaria, Thecophora modesta, and T. occidensis, although further collecting in these regions may add to this list. Present records are insufficient to determine if any conopid species are truly cordilleran or coastal in distribution.

**Ecological Associations.** Host records are scarce. However, some generalizations regarding ecological roles can be drawn. Hosts appear to be determined roughly along generic lines within Conopidae. Large species, especially those of *Physocephala* and *Physoconops*, parasitize larger bees and wasps as hosts such as Apidae (*Apis*, *Bombus*) and Crabronidae (*Bembix*). Smaller conopids, including *Dalmannia*, *Myopa*, and *Thecophora*, are possibly limited to smaller bees as hosts (Andrenidae, Halictidae). There are not enough host records to estimate host patterns for *Zodion* species.

Dalmannia and Myopa appear to be the early emerging genera within Conopidae as adult records are limited to April through June. For all other genera, adults emerge from June to September. There evidently are no phenological differences among species within a given genus, but more records are necessary to clarify this point.

Plant associations as recorded may be a by-product of conopid phenology. Most species of Conopidae frequent many different families of plants. The only discernible pattern appears to be the early emergence of *Myopa* coordinated with some early-blooming plants including willows (Salicaceae). Of course, these plant associations do not necessarily indicate that the flies are pollinating the plants visited.

Hilltopping is observed in all genera of Conopidae. Whether this behaviour is obligate, facultative, or geographically determined in any species requires further observations. Accessible hilltop locations may provide valuable data on this question.

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# Supercolonies of the invasive ant, *Myrmica rubra* (Hymenoptera: Formicidae) in British Columbia, Canada

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# **ABSTRACT**

Levels of intra-specific aggression between workers and mtDNA sequence comparisons were used to demonstrate that the non-native, invasive ant, *Myrmica rubra* L. has formed supercolonies in southwestern British Columbia. Ants from most, but not all, infested areas act aggressively towards ants from other areas but workers from widely separated locations within two of the largest areas show little aggression towards each other. Comparisons of COX1 mtDNA nucleotide sequences suggest that formation of different supercolonies may have followed possible divergence after a single initial introduction to the province.

Key words: invasive, super-colony, aggression

# **INTRODUCTION**

Worldwide, over 150 species of ants have been introduced into new environments (McGlynn 1999) but a small number have become invasive, i.e., have reduced native ant biodiversity (Holway et al. 2002). Naumann and Higgins (2015), Gargas et al. (2007), and McPhee et al. (2012) have all reported that recently-established populations of Myrmica rubra L. in northeastern North America and the Pacific Northwest have all the characteristics of an invasive ant. In southwestern British Columbia M. rubra populations have dramatically decreased the incidence and abundance of previously established ants in three different plant communities: a well-drained riparian zone dominated by cottonwood (Populus balsamifera subsp. trichocarpa (Torrey and Gray) Brayshaw; Salicaceae) and Scotch broom (Cytisus scoparius (Linnaeus) Link; Fabaceae); a moister, more shaded community, dominated by red alder (Alnus rubra Bongard; Betulaceae), and two exotic blackberries, Himalayan blackberry (Rubus discolor Weihe and Nees; Rosaceae), and evergreen blackberry (Rubus laciniatus Willdenow); and grassy fields (Naumann and Higgins 2015). They also occur at unusually high densities compared to previously established species. Myrmica rubra represented more than 99.99% of the total ant fauna caught in the infested areas, and their capture numbers in the plant communities ranged from 10 to 1300 times the total number of all ants collected in corresponding M. rubra-free areas. The numbers of several other taxa of insects and non-insect arthropods were also reduced where M. rubra was present (also reported by Gargas et al. 2007).

Myrmica rubra is native to Northern Europe and western Asia and was first documented in North America in Massachusetts in 1908 (reviewed in Groden et al. 2005). It has now been reported in all Canadian provinces east of Manitoba and in at least six northeastern United States, and Washington state. Most of the reports are from within the last ten years, suggesting that the North American populations are expanding

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(Wetterer and Radchenko 2011). North American populations have not been observed to produce flying females (Hicks 2012), so spread is suspected to occur via the transport of garden products and by colony budding. The species likely established in southwestern British Columbia over 20 years ago but went relatively unnoticed for several years (Higgins 2013). *Myrmica rubra* comes to the attention of the public mostly because of a painful sting and high densities. Stinging is an unusual feature among the ant species listed from British Columbia (Naumann et al. 1999) and can make yard and garden work difficult and cause distress for pets. There is also concern that these ants may be interfering with the successful nesting of some birds (Higgins 2013). Robinson et al. (2013) estimated that the economic cost of this species in British Columbia could reach \$100 million/year if it spreads across its potential range in the province.

The formation of supercolonies may contribute to the ability of invasive ant species to monopolize resources. An ant supercolony can be defined as a population of ants that exists over a large, contiguous area and in which ants move freely between nests and appear to show no aggression to conspecifics. (Haines and Haines 1978; Moffett 2012). Ants within a supercolony are so numerous that is impossible for all members of the colony to interact in their lifetime (Pedersen et al. 2006).

Holway et al. (2002) give a thorough review of the reported interactions between invasive and native ants worldwide. Especially common are reports that invasive species show greater efficiency at exploiting resources. Better resource utilization could be due to a larger force of workers, i.e., more scouts and more foragers to recruit, and/or physical aggression toward other species at a food item (Garnas et al. 2014). Supercolonies are typically seen only in non-native populations and are typified by being multiple-nested, multiple-queened, and lacking distinct behavioural boundaries among physically separate nests. This sort of colony organization has allowed a small number of non-native invasive species such as the Argentine ant, Linepethema humile (Mayr) (on all continents except Antarctica); the little fire ant, Wasmannia auropunctata (Roger) (in Africa, the Americas, and some Pacific islands); and the African big-headed ant, Pheidole megacephala (Fabricius) (all continents except for Antarctica), to attain high local abundances and consequently to dominate entire habitats (Holway et al. 2002). The 'Large Supercolony' of L. humile in California spans 1,000 km in distance (Moffett 2012). An apparent absence of intraspecific aggression within such supercolonies may free up time and energy for other uses.

The purpose of this study was to gain a better understanding of how *M. rubra* has come to dominate its new habitat in BC by determining if supercolony formation has occurred. The number of behviourally and genetically distinct colonies may give insights into whether there has been a single successful introduction or more than one.

# **METHODS AND MATERIALS**

This study was carried out using ants from seven geographically distinct populations of *M. rubra* in southwestern BC. Prior to this study, there was no indication of whether those populations are the product of a single introduction or more than one. The frequency of aggressive interactions between workers was used to determine whether ants from the different areas, and from within them, treated each other as nestmates. The degree of genetic similarity of workers from the same seven areas was estimated by comparing nucleotide sequences of the mtDNA gene for cytochrome oxidase subunit I. It was hoped that this would provide a molecular level confirmation of any patterns of relatedness suggested by the behavioural data.

i) Sourcing and rearing the ants. Colonies of several hundred workers and at least two queens were collected in the last week of May and first week of June 2014 from nests within seven areas of infestation: Sea Island (Richmond), Fraser River Park (Vancouver), Inter River Park (North Vancouver), University of BC (Point Grey), Chilliwack, south Burnaby, and Oak Bay on Vancouver Island. It is unlikely that *M*.

rubra has been established in each area for the same length of time. The source colonies for this study were not identical in size, and not all workers were captured but the assumption was made that this would not have an important influence on the behaviour or individual workers. Each captured colony was maintained, in a laboratory, in a soil-free, 34 x 23 x 8 cm lidded, plastic tub which contained an aluminum foil-covered, 13 x 9.5 x 5 cm plastic container that acted as the nest. The internal box contained multiple folds of moist paper towel. Each colony was given a supply of water (a water-filled test tube stopped with a cotton ball), 1:1 honey water mixture, apple slices, and recently killed meal worms, and kept on a 12:12 h light-dark cycle.

ii) Inter-nest worker aggression between infestation areas. The level of aggression in interactions between ant workers of the same species has often been used as a proxy for levels of genetic difference – i.e. as a method of determining nest mate recognition – and many types of bioassays have been reported (Roulston et al. 2003). The recognition system that ants use for identification with a colony and rejection of aliens is based on shared cues, typically a colony-specific odour blend generated by queens or workers (d'Ettorre and Lenoir 2010), although food and other environment factors can have an influence (Liang and Silverman 2000). Our aim was to use the level of aggression between workers from the seven different areas as a correlate of the degree of genetic similarity. To minimize the confounding effects of foods and odours brought into the lab colonies from their original environments, all colonies were maintained for at least one week prior to being used for bioassays. It was assumed that several weeks in the lab would not diminish the tendency for ants from different colonies to fight, which we defined as ants locked together as they grasped each other with their mandibles.

Methods to measure the level of intraspecific aggression were similar to Roulsten et al. (2003) and are summarized as follows. Sets of workers from each area of infestation were matched with workers from a nest from each of the other areas. There were eight to ten replicates (trials) for each pair. For each trial, five foragers from each of two colonies were transferred to a fluon-coated 250 ml glass beaker which acted as a neutral arena. The number of ants engaged in fights was recorded during five-second scan surveys carried out once every minute for 10 minutes. For comparisons, we used the average (of 10 observations) percentage of ants involved in fights at one time across all colony pairs. For half of the replicates, the first five ants into the arena came from one of the colonies within each pair; for the other half, they came from the second colony. Controls consisted of bioassays of two groups of five ants from the same colony.

The aggression bioassays were repeated a minimum of four weeks after capture, i.e., during the second week of July, 2014. This was meant to test both that the initial one-week latent period in the lab had been long enough to remove the effects of environment, and that maintenance in the lab did not result in loss of aggression. This length of delay was chosen because laboratory colony populations were beginning to decline at that time. Colonies collected from Oak Bay were not included in this particular test because of diminished worker numbers.

Within Infestations. The level of inter-nest aggression was also measured between nests from within two of the largest areas of infestation, Sea Island in Richmond and Fraser River Park in Vancouver. At Sea Island, four nests were collected at approximately 500 m intervals along a 2 km transect line; at Fraser River Park, three nests were collected approximately 300 m apart along a 1 km transect line. As before, the nests were reared in the laboratory, as described above. Aggression bioassays were carried out one week after the establishment of the nests; n = 6-10 for each pairing.

ii) The Genetic similarity of ants from different M. rubra populations. Differences in mtDNA nucleotide sequences were measured as a way to determine if there had been a single successful introduction of *M. rubra* into BC, or more than one. It was also hoped that mtDNA differences could allow for discernment of ants from different areas, i.e., different possible supercolonies. All mtDNA samples were collected from workers from the nests used for the aggression bioassays.

DNA was extracted from ants using a modified procedure of Schlipalius et al., 2001. This procedure allowed for the use of whole insects combined with particular primers that limit the possibility of contamination with microbial DNA. Individual frozen ants were removed from storage at  $-80^{\circ}$ C and immediately crushed in the bottom of a 1.5 ml Eppendorf tube with an extraction buffer consisting of 30µl of boiling 5% Chelex in TE. Each tube was then placed into a boiling water bath for 15 min and centrifuged at 13,000 rpm for 10 min. 20 µl of the supernatant was removed from each sample, and put into storage at  $-20^{\circ}$ C for later use as template DNA in PCR reactions.

The PCR primer pair LC1490 and HCO2198 (Folmer et al. 1994) were used for the amplification of a 710 bp partial coding sequence of mitochondrial cytochrome oxidase subunit I (COX I). Primers were custom synthesized by INVITROGEN/ Life Technologies <sup>TM</sup>. PCR was done using 2 μl of PCR buffer, 1 μl of 1 μM of primer solution, 1 μl of Taq polymerase (Amplitaq, from Life Technologies), 1 μl of a 2 mM dNTP, and 1 μl ant DNA, with dH<sub>2</sub>O added to a total volume of 20 μl. PCR was run on a Techne Techgene thermal cycler. The program settings were: initial denaturation at 95°C for 2 minutes; 30 cycles of the following: 30 seconds at 94°C, 45 seconds at 50°C, 2 minutes at 72°C, and a final extension for 5 minutes at 72°C. Successful amplification of single 710 bp DNA from all ants was confirmed by agarose gel electrophoresis (data not shown) and purified using a QIAquick PCR Purification Kit from QIAGEN. DNA was sequenced using the Sanger method on an Applied Biosystems 3730 DNA analyzer at the NAPS Unit at the University of British Columbia, Vancouver, BC.

Formica sinensis. Wheeler cytochrome oxidase subunit partial coding sequence (Accession EU983580) was used as an outgroup to determine the order of descent among DNA sequences. Phylogenetic analyses were done using MEGA7 (Kumar et al. 2016). Sequences were imported into MEGA7 as fasta files and MUSCLE was used to generate an alignment using the ALIGN CODONS option. Phylogenetic trees were generated using the Maximum Likelihood Estimation (Tamura 1992; Felsenstein 1985). Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. The analysis involved 17 nucleotide sequences. Codon positions included were 1<sup>st</sup>+2<sup>nd</sup>+3<sup>rd</sup>+Noncoding. All positions with less than 95% site coverage were eliminated – i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 438 positions in the final dataset.

## RESULTS

Aggression Bioassays. With the exception of one pairing of localities, ants from nests originating in different areas of southwestern BC showed high levels of workerworker aggression (Table 1). This included ants from Sea Island and Fraser River Park, which are separated only by an arm of the Fraser River. The exception was a lack of aggression observed when ants from Inter River Park in North Vancouver encountered ants from Point Grey (University of British Columbia, Vancouver). The patterns of fighting between workers from different localities did not change when tested again a further four weeks after the nests were brought into the laboratory (Table 2).

There was comparatively little fighting between ants from nests *within* the Sea Island or Fraser River Park *M. rubra* populations, even when nests were as much as 2 km apart (Table 3).

Genetic Comparisons. Figure 1 shows that the nucleotide sequences of the COXI subunits of the ants from the different outbreak areas fell into two groups. The North Vancouver and Point Grey ants were within the same group. Different samples from Fraser River Park in Vancouver fell within either group.

#### Table 1

Mean percentage (± SD) of ants from different pairs of colonies engaged in fights after one week of laboratory rearing. The ants were from nests in seven different areas of southwestern British Columbia. FR Park = Fraser River Park, Vancouver. Data with different superscripted letters are significantly different (p < 0.05; ANOVA and LSD multiple comparison tests; F = 104.6; df = 20; P<0001. Same-nest comparison data were not included in the statistical analysis).

,	Oak Bay	Burnaby	Chilliwack	UBC	N Van	FR Park	Sea Is
Sea Is	74(12) <sup>e</sup>	51(22) <sup>c</sup>	72(28) <sup>ef</sup>	79(24) <sup>efg</sup>	44(26) <sup>b</sup>	62(19) <sup>d</sup>	0
FR Park	87(7)hi	81(13) <sup>fgh</sup>	83(12)gh	84(5)gh	80(13) <sup>efg</sup>	0	
N Van	78(12) <sup>efg</sup>	52(6) <sup>c</sup>	80(13) <sup>efg</sup>	$0(0)^{a}$	0		
UBC	92(27)efg	54(20)°	90(7) <sup>i</sup>	0			
Chilliwack	55(16) <sup>c</sup>	83(6)gh	0				
Burnaby	79(10) <sup>efg</sup>	0					
Oak Bay	0						

#### Table 2

Mean ( $\pm$  SD) percentage of ants from different pairs of colonies engaged in fights after six weeks of laboratory rearing. The ants were from nests in six different areas of southwestern British Columbia. FR Park = Fraser River Park, Vancouver; nests from a seventh locality (Oak Bay) were not tested at the six week interval. Data with different superscripted letters are significantly different (p < 0.05; ANOVA and LSD multiple comparison tests; F = 94.7; df = 12; P<0.0001)). \*Insufficient ants.

	Burnaby	Chilliwack	UBC	N Van	FR Park	Sea Is
Sea Is	69(20) <sup>de</sup>	53(21)°	61(16) <sup>d</sup>	64(14) <sup>d</sup>	21(15) <sup>b</sup>	0
FR Park	51(18) <sup>d</sup>	73(10) <sup>ef</sup>	79(4) <sup>f</sup>	77(11) <sup>f</sup>	0	
N Van	43(23)°	83(7.5) <sup>f</sup>	3(8) <sup>a</sup>	0		
UBC	*	*	0			
Chilliwack	63(21) <sup>d</sup>	0				
Burnaby	0					

# **DISCUSSION**

Invasive populations of *M. rubra* have formed at least two large, multi-nest supercolonies in BC, and it is reasonable that the same phenomenon has occurred in the other distinct areas of infestation. The one on Sea Island is several kilometers across and, as individual nests are often less than 5m apart, must contain thousands of nests and millions of individual ants. This type of colony organization may be contributing to the displacement of native ants and other epigaeic species that was reported by Naumann and Higgins (2015).

Based on aggression bioassays, most of the major outbreak areas of *M. rubra* in southwestern BC represent different super-colonies, but workers from UBC and North Vancouver interact as if they are nest mates, suggesting either a relatively recent common origin, or that one site was the source of the founding population of the other.

#### Table 3

Mean (± SD) percentage of ants from different pairs of colonies within the same outbreak areas; SI = Seal Island; FP = Fraser River Park) that engaged in fights after one week of laboratory rearing. The ants were from nests separated by approximately 500 m (SI) or 300 m (FR) intervals along a transect line.

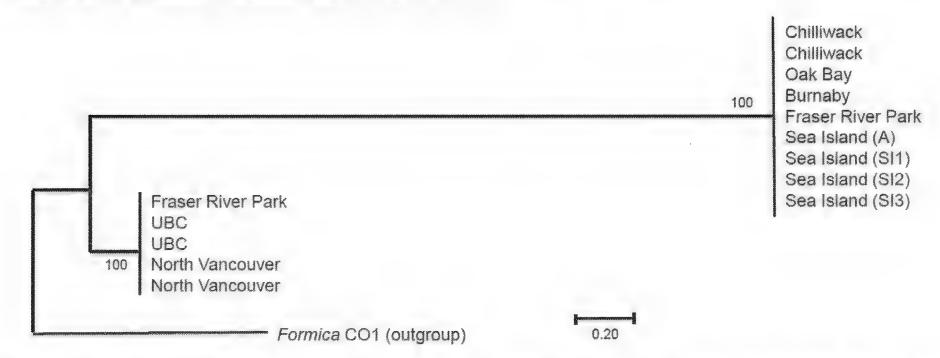
	SI1	SI2	SI3	SI4		FP1	FP2	FP3	
SI4	2(7)	3(6)	0	0	FP3	0	4(6)	0	
SI3	3(6)	2(6)	0		FP2	0	0		
SI2	2(7) 3(6) 13(10) 0	0			FP3 FP2 FP1	0			
SI4	0								

Levels of aggression between ants from different colonies are frequently used as a proxy for levels of genetic difference (Roulsten et al. 2003). In this study, patterns of aggression did not change markedly after a minimum of four weeks in the lab, suggesting that it was not chemical cues associated with the original environments that led to recognition of individuals from different locations, but rather colony-specific odor blends generated by queens or workers (d'Ettorre and Lenoir 2010), and likely to be genetically based.

We did not find enough molecular diversity in COXI to be able to distinguish between different populations of *M. rubra* in southwestern BC but the significant separation into two groupings, with ants from Fraser River Park common to both, suggest that an original introduction into BC may have occurred near there, and that divergence of this subunit occurred later. The observation that ants from within the Fraser River outbreak treat each other as nest mates argues against two genetically unique introductions. The similarity of the COXI sequences of the non-aggressive ants from UBC and North Vancouver provides further evidence that those two groups of ants are particularly closely related. Hicks et al. (2012), also using mtDNA, reported evidence that *M. rubra* populations on Newfoundland have come from at least four distinct sources, including the UK and the Northeastern USA. We do not yet have enough data to speculate on the possible source of the *M. rubra* populations in BC.

Possible mechanisms for the superior competitive abilities of invasive ant populations include direct aggression, superior recruitment to resources, and higher activity levels. Garnas et al. (2014) reported that *M. rubra* shows both higher levels of recruitment and aggression towards native ant species in Maine, USA; foragers consistently discover baits faster and displace foragers from native species. Foragers from highly populous supercolonies with many dispersed nests would have an advantage at discovering, recruiting to, and exploiting food resources. For example, supercolony-forming *L. humile* have been reported to be more numerous than other species in the same area, and to be a superior interference competitor that displaces native species from contested baits, often via direct physical aggression (Human and Gordon 1996). In addition, lack of aggression between workers over large areas could leave more time and energy for foraging. *Linepithema humile* for example, maintains higher colony activity levels,

forages for longer periods each day, and recruits in greater numbers to food resources than native species (Human and Gordon 1996).



**Figure 1.** Molecular phylogenetic tree of the COXI subunit of mtDNA from *M. rubra* workers from different outbreak areas of British Columbia. The tree with the highest log likelihood (-1824.1574) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

The proximate and ultimate causes of supercolony formation remain inconclusive. According to Holway et al. (2002), the phenomenon is more common among ant species that are non-native and have become invasive in their newly established environments. They also tend to show relatively small size, omnivory, and a tendency towards multiple queen nests. On the other hand, most of these species exhibit similar life histories in their native ranges (Moffett 2012), and at least one other ant species, Liometopum occidentale Emery, may form large (at least one km in diameter), habitat-dominating supercolonies within its home range (Wang et al. 2010). Failure to form large colonies in those areas may be due to constraints by other native species that are aggressive and effective competitors. In other words, it is the release from those competitors in a new region that allows for the formation of supercolonies (Moffett 2012). formation in M. rubra, as in other supercolony-forming species, may also be related to the fact that virgin queens in North America do not carry out mating flights (Hicks 2012), although they do in their home range. Instead, North American queens mate at or near the nest and then travel a short distance, with a group of workers, to found a new nest. Infestations thus expand relatively slowly via colony budding, and jump to new areas, likely through human activities like the transport of infested nursery products. It is possible that lack of contact with conspecifics from other colonies inhibits queen mating flights or fails to stimulate them. If there are no intraspecific competitors in an adjacent area, why risk a mating flight when territory that is likely to be suitable lies right next door? Also, the success of incipient colonies is likely to be higher if the queen is not alone, and if the number of founding workers is greater (reviewed in Holway et al. 2002).

Although it is now possible to add *M. rubra* to the list of invasive ant species that share a suite of behavioural features such as supercolony formation, much work needs to be done to resolve both the details of the *M. rubra*'s establishment in different areas of North America, and the general mechanisms that lead to the formation of ant supercolonies. Do some ants become ecologically dominant because they form supercolonies or does the monopolization of resources by certain species lead to supercolony formation (Hölldobler and Wilson 1977)?

# ACKNOWLEDGMENTS

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#### **SCIENTIFIC NOTE**

# First record of *Aedes (Ochlerotatus) spencerii* (Theobald) (Diptera: Culicidae) in the Yukon

# DANIEL A.H. PEACH1

Aedes spencerii (Theobald) (Diptera: Culicidae) is a small- to medium-sized mosquito with characteristic alternating dark- and pale-scaled wing veins that is a common inhabitant of grassland areas (Wood et al. 1979). It has two subspecies: Ae. s. spencerii and Ae. s. idahoensis (Darsie and Ward 2005). Males and females have been observed nectar feeding on catkins of willow (Salix sp.) (Knab 1907) and goldenrod flowers (Solidago sp.) (Philip 1943), and females are known to take blood meals from avian and mammalian hosts (Rempel et al. 1946). It has very rarely been found carrying western equine encephalitis (McLintock et al. 1970) and West Nile virus (Anderson et al. 2015).

Ae. spencerii overwinters in the egg stage and is one of the first mosquito species to emerge in the spring (Wood et al. 1979). Larvae are found in many habitats, including pools of water formed by heavy rainfall, floodwater, or snow-melt (Belton 1983). Larvae can develop rapidly at low temperatures, with pupae collected in mid-April near Ottawa, Ontario, when the larvae of other Aedes spp. were only half grown (Wood et al. 1979). One or more generations will develop per year; however, suitable drying and subsequent flooding of oviposition sites is necessary for the development of additional generations beyond the first (Wood et al. 1979). In some areas, females have emerged as late as September when these conditions are met (Philip 1943).

The known distribution of Ae. spencerii ranges roughly from the Great Lakes to central British Columbia, and from Colorado to Churchill, Manitoba. Scattered populations also exist in Ottawa, Ontario, as well as in the states of New York, New Jersey, and Oklahoma (Darsie and Ward 2005).

Two adult female mosquitoes attempting to bite the author were collected and placed in ethanol on August 28, 2016, near Lake Creek campground in the Shakwak valley of the southwest Yukon. The specimens were identified using the keys of Darsie and Ward (2005) and Thielman and Hunter (2007) as Ae. s. spencerii (Fig. 1) and Aedes sticticus. The collection site was mostly valley-bottom muskeg and riparian area with vegetation present, including black spruce (Picea mariana), unidentified mosses, lingonberry (Vaccinium vitis-idaea), willow (Salix sp.), and Labrador tea (Rhododendron groenlandicum). Many snowshoe hares (Lepus americanus) and American red squirrels (Tamiasciurus hudsonicus) were observed in the area. Growing nearby were patches of fireweed (Chamerion angustifolium) and stands of aspen (Populus tremuloides). Numerous small bodies of water were present in the vicinity, as was additional vegetation that the author did not note at the time. The southwest Yukon is home to patches of grassland, particularly in the Kluane Lake area (Laxton et al. 1996; Conway and Danby 2014). This Ae. s. spencerii specimen may have originated from some such nearby patch of grassland, or possibly from grassy patches along the margins of the nearby Alaska Highway.

Mosquito collecting in the Yukon and the species recorded there were last reviewed by Belton and Belton (1990). This is the first record of *Ae. spencerii* in the Yukon and confirms the presence of *Ae. sticticus*, which was previously uncertain (Belton and

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Belton 1990). Both specimens have been deposited with the Beaty Biodiversity Museum at the University of British Columbia.

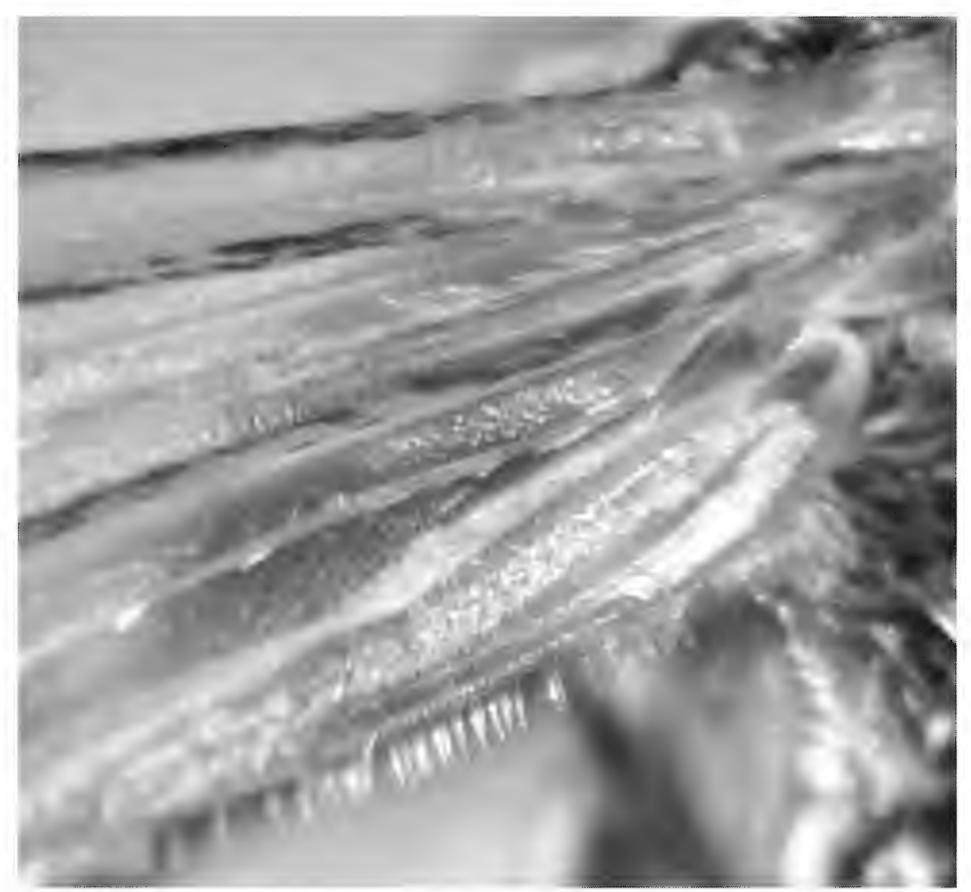


Figure 1. Close-up of characteristic alternating dark- and light-scaled wing veination of Ae. spencerii.

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#### **SCIENTIFIC NOTE**

# Cold requirements to facilitate mass emergence of spruce beetle (Coleoptera: Curculionidae) adults in the laboratory

# K. P. BLEIKER<sup>1</sup> and K. J. MEYERS

ABSTRACT— The spruce beetle, *Dendroctonus rufipennis* Kirby (Coleoptera: Curculionidae, Scolytinae), is a native disturbance agent of spruce (*Picea* spp.) forests in North America. Based on field observations, it is widely accepted that new adults must overwinter regardless of the length of the life cycle. We tested the effect of different lengths of time at 4° C on spruce beetle emergence. Our objective was to determine a protocol for rearing spruce beetle to facilitate laboratory-based research. We found that for spruce beetles from north—central Alberta and southern British Columbia, 70 d at 4° C led to rapid mass emergence of adults. Adults also emerged in the absence of a cold period, but over an extended period of time.

Key words: spruce beetle, *Dendroctonus rufipennis*, bark beetle, rearing, emergence, diapause, overwintering

The spruce beetle *Dendroctonus rufipennis* Kirby (Coleoptera: Curculionidae, Scolytinae) is a native disturbance agent of spruce (*Picea* spp.) forests in North America. Large-diameter weakened and injured trees, fresh-cut stumps, cull logs, windthrow, and drought-stressed trees are the preferred hosts (Dyer and Taylor 1971; Safranyik 2011; Hart *et al.* 2013). Populations may build up in these hosts and spill over into mature healthy standing trees once the preferred hosts have been depleted (Safranyik *et al.* 1983; Safranyik 2011). Controlled rearing of spruce beetle in the laboratory may facilitate experiments aimed at understanding factors affecting the population dynamics and control of this economically important insect.

Bark beetles are easily reared in logs in the laboratory; however, some species require a cold period to complete their life cycle (Ryan 1959). A two-year life cycle is common throughout much of spruce beetle's range; under warmer conditions, the life cycle may be completed in one year and, in areas with cool, wet summers, the life cycle may take as long as three years (Massey and Wygant 1954; Knight 1961; Berg et al. 2006; Werner et al. 2006). Adult beetles emerge from overwintering in the natal host to attack new trees in the spring, usually in late May or June, although attacks can occur throughout the summer. Females bore into the inner bark, where they are joined by a male, mate, construct egg galleries, and lay eggs. The majority of the life cycle is completed under the bark, with larvae mining the inner bark before pupating and eclosing to new adults. Cool temperatures trigger what has been described as a facultative larval diapause in the two- and three-year life cycles, although the conditions that trigger the diapause and instar sensitive to the cue might vary geographically (Dyer and Hall 1977; Hansen et al. 2011). Based on numerous field observations, new teneral adults always overwinter once, regardless of the length of the life cycle, and it is widely accepted that new adults must overwinter to become sexually mature (Massey and Wygant 1954). Although it has not been demonstrated experimentally, it is now widely accepted that spruce beetle has an obligatory adult diapause (e.g., Raffa et al. 2015). New teneral adults overwinter in windthrow or standing trees where they developed; however, a proportion of beetles in standing trees may emerge in the fall, move to the base of the same tree, and bore under the bark where they overwinter in groups insulated from cold winter temperatures and

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woodpecker predation by the snow pack (Massey and Wygant 1954; Knight 1961; Fayt et al. 2005).

In this paper, we report the effect of different lengths of time at 4° C on spruce beetle emergence. Our objective was to determine a protocol for rearing spruce beetle in the laboratory that would lead to mass emergence of new adults. We selected 4° C as our treatment temperature, because it met the adult cold diapause requirements of Douglas-fir beetle *D. pseudotsugae* Hopkins (Ryan 1959) and it could be easily achieved with a regular refrigerator. We used two distant populations in the trial: Grande Prairie (GP) in north—central Alberta (N54.860800, W118.713567; 649 m) and Placer Creek (PL) in southern British Columbia (N49.15332, W120.47453; 650 m).

Spruce bolts (35-cm-long logs) were cut from a recently infested tree at each site and transported to the laboratory at the Pacific Forestry Centre, in Victoria, British Columbia. Five bolts were cut at GP in late-May 2013 and four bolts were cut at PL in mid-June 2013. The GP tree was likely pure white spruce (Picea glauca (Moench) Voss) and the PL tree was likely pure Engelmann spruce (P. engelmannii Parry ex Engelm.), although introgression between the species beyond their reported ranges is possible (see Maroja et al. 2007 and references therein). Parent beetles were constructing galleries and laying eggs at the time the material was collected. The bolts were placed vertically in emergence cages and held in a room at approximately 22° C with a photoperiod of 16 light:8 dark until 29 August 2013, when the presence of fully darkened teneral adults was confirmed by removing several small pieces of bark. As developing larvae were reared at 22° C, the facultative larval diapause was not triggered. At this time, one bolt from each population was left at 22° C and the other bolts were placed in a walk-in cold room at 4° C. The cold room was dark, except when someone transferred material in or out of it. One bolt from each population was removed after 15 (GP only), 30, 50 and 70 days in the cold room and placed back at 22° C. Henceforth in this report, we use the population code followed by the number of days at 4° C to refer to the different treatments. For example, GP30 and GP0 refer to insects from Grande Prairie, which were held at 4° C for 30 d and 0 d, respectively, while PL30 and PL0 refer to insects from Placer Creek receiving those treatments.

Emerging beetles were collected at least three times per week. Average daily emergence was calculated by dividing the number of beetles collected by the number of days since the last collection. After at least 10 days of zero beetles emerging from a bolt, the bark was removed and any teneral adults remaining under the bark were counted and recorded as alive or dead.

The majority of beetles in all treatments emerged. Eighty-nine percent or more of the beetles emerged, with two exceptions: PLO, 28% of the total number of beetles failed to emerge and were found dead under the bark; and GP50, 18% of the total number of beetles failed to emerge and most of these beetles were found alive under the bark (Table 1). For rapid mass emergence, the best cold treatment was 70 d for both beetle populations (Figure 1). Beetles subjected to 70 d of cold had a notable increase in emergence within 10 d after the cold treatment was terminated, and the vast majority of beetles emerged rapidly within a 10-d period (Figure 1). Beetles in the 50-d treatment also emerged soon after being removed from the cold room. However, after approximately 65% and 80% of the beetles had emerged from PL50 and GP50, respectively, emergence plateaued for almost two weeks (Figure 1). The remaining beetles emerged from PL50 but, in the case of GP50, the bolt was peeled after 10 d of no emergence and 17% of the total number of beetles were still under the bark and were alive (Table 1); these beetles may also have emerged given more time. Our results are similar to what Ryan (1959) reported for Douglas-fir beetle, which also overwinters as a teneral adult: 90 d of cold treatment was more effective than 50 d for triggering emergence. He also tested a number of cold temperatures from 0.5 to 12.5° C, and found that 6.6° C was the optimum cold temperature, although emergence following 90 d at 6.6 and 4° C were similar.

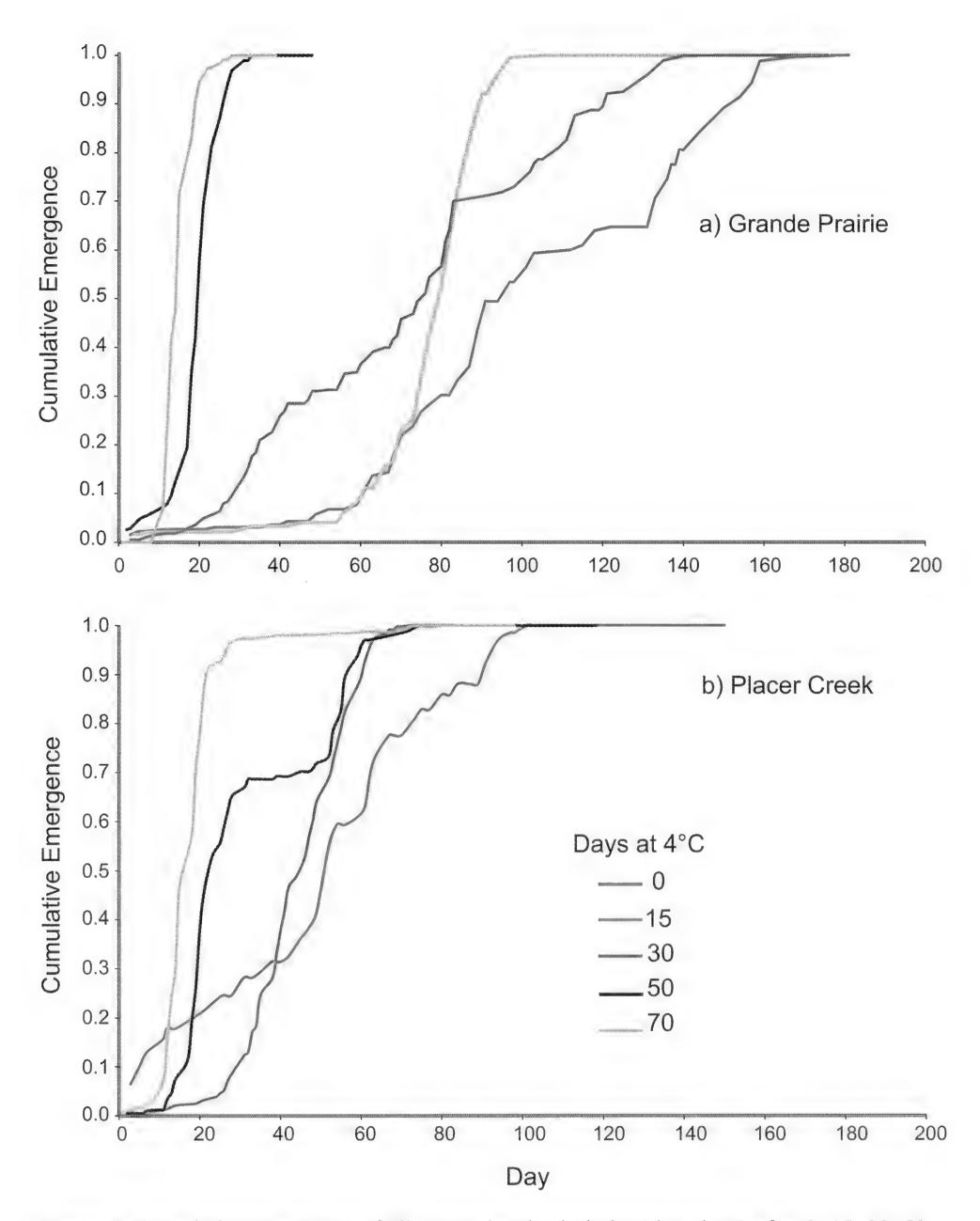
Table 1

Percentage of spruce beetles emerging from bolts (short logs) after 0, 15, 30, 50 or 70 d at 4° C. Insects were fully darkened teneral adults when cold treatments were initiated. After at least 10 d of no emergence, the bark was removed and the number of live and dead beetles remaining under the bark was recorded. Bolts were cut at two sites: Grande Prairie (GP), in north–central Alberta, and Placer Lake (PL), in southern British Columbia.

Site	Days at 4° C	Total Beetles (n)	Emerged (%)	Dead (%)	Unemerged Live (%)
GP	0	98	96	4	0
	15	129	94	6	0
	30	272	94	6	0
	50	328	82	1	17
	70	169	99	1	0
PL	0	117	72	28	0
	30	259	95 -	5	0
	50	281	98	2	0
	70	282	89	11	0

Emergence tended to be slower from bolts receiving 30 d or less of cold treatment (Figure 1). Emergence from both GP30 and PL30 did not notably increase until after 21 d at 22° C, and emergence was also delayed after the cold treatment was terminated for GP15, although once it started it was relatively rapid (Figure 1). In the absence of a cold treatment, beetles emerged over an extended period of time (over 100 d) and it took longer for beetles to start emerging from GP0 than from PL0. The new adults may have been at different levels of maturation, as the populations may have received slightly different degree day accumulations based on temperatures in the field and when the trees were infested and cut. In addition, there may be geographic variation in the developmental rates of bark beetles (Bentz et al. 2001) or the proportion entering diapause (McKee and Aukema 2015). We also cannot determine if beetles were emerging to overwinter at the base of the tree or to disperse to attack a new host tree. Ryan (1959) determined that Douglas-fir beetles in diapause had underdeveloped reproductive organs. We did use a number of the new adults emerging from GP70 and PL70 in another experiment. These beetles entered fresh bolts and successfully reproduced, indicating they were sexually mature upon emergence; however, we did not use beetles from the other cold treatments, so their level of sexual maturity remains unknown.

Our results indicate that a cold period promotes the rapid mass emergence of new spruce beetle adults. In the absence of a cold period, 72% of PL beetles and 96% of GP beetles still emerged, although they emerged slowly over approximately 100 d. We have demonstrated that 70 days of cold treatment at 4° C is sufficient to trigger mass emergence of adult spruce beetles. This treatment can be used to rear insects in the laboratory, thereby facilitating experimental research.



**Figure 1.** Cumulative emergence of all spruce beetles in bolts (short logs) after 0, 15, 30, 50 or 70 d exposure to 4° C before being held at 22° C (day 0). Bolts were cut at two sites: a) Grande Prairie (GP), in north–central Alberta, and b) Placer Lake (PL), in southern British Columbia.

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#### **SCIENTIFIC NOTE**

# Production of epicormic buds by Douglas-fir in central British Columbia, Canada, following defoliation by western spruce budworm (Lepidoptera: Tortricidae)

#### LISA M. POIRIER1

Western spruce budworm, *Choristoneura freemani* Razowski (= *C. occidentalis* Freeman), is an important defoliator of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, in southern British Columbia (Maclauchlan *et al.* 2006). Defoliation by larvae can result in reduced tree growth, top-kill and occasional tree mortality (Alfaro *et al.* 1982). Larvae feed on the new foliage of all ages of trees; mortality is most common in immature and suppressed understorey trees (Maclauchlan and Brooks 2009), but repeated, severe defoliation can kill larger trees.

Flushing of buds late in the season is a proposed mechanism by which conifers can compensate for defoliation (Piene 1989), and increased late bud production may explain why some species or individuals experience greater survival and faster recovery following defoliation (Piene and Eveleigh 1996). The terminology used in the literature for these late-flushing buds varies, but Meier *et al.* (2012) recommend calling them epicormic buds, while sequential buds are those formed during shoot elongation.

The most recent western spruce budworm outbreak in British Columbia extended farther north than had been observed previously. Near the northern edge of that outbreak, my observations suggested that defoliated Douglas-fir had fewer flushed epicormic buds at the end of the summer than might have been expected farther south. At higher latitudes, a short growing season and early frosts may reduce the ability of trees to compensate for defoliation by producing epicormic buds.

In 2010, two-year-old *P. menziesii* var. *glauca* seedlings were obtained from Pacific Regeneration Technologies, Inc. in Red Rock, B.C. Seedlings were planted individually in conical pots of all-purpose potting mix on May 17, when the earliest sequential buds on local Douglas-fir trees began to swell. Third- to fifth-instar western spruce budworm larvae were collected from two sites north of Williams Lake, B.C. (52.266° N, 122.285° W and 52.471° N, 122.434° W) on June 16. Larvae were kept on live foliage in large plastic bags and transported to the University of Northern B.C. (UNBC) in Prince George, B.C. (53.893° N, 122.816° W), then transferred to the experimental seedlings within 48 hr. Each seedling had 18–22 sequential buds at approximately the same stage of development as those of Douglas-fir on campus.

Five treatments (n = 24 each) were applied on the same day as follows.

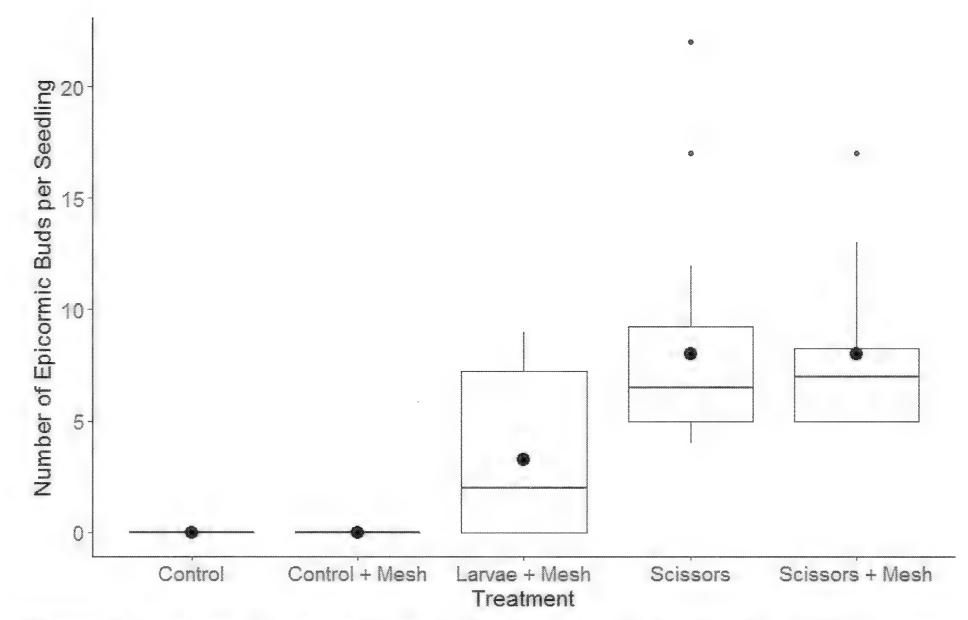
- 1. Control: No further manipulation of seedlings.
- 2. Control+mesh: A white, polyester-netting (BioQuip Products Inc.) cylinder was secured with elastic bands over each seedling.
- 3. Scissors: All sequential buds were removed at the base with fine scissors.
- 4. Scissors+mesh: Sequential buds were removed with scissors, and a netting cylinder was secured over each seedling.
- 5. Larvae+mesh. A netting cylinder was secured over each seedling, and 10 larvae were added. The larvae on each seedling ranged from approximately third to fifth instar, representing the range and distribution of larvae collected at field sites.

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A sixth treatment, larvae on seedlings without mesh, was not possible due to the risks of releasing insects in an area where they are not currently found. Seedlings were randomized within racks and placed outside in the compound of the Enhanced Forestry Laboratory at UNBC. They were watered daily, and pupae were removed twice weekly.

Once all larvae had pupated or died, the mesh bags were removed, and flushing epicormic buds were counted for each seedling. Due to heteroscedasticity and small sample size, treatments were compared using a Kruskal–Wallis rank sum test and a Nemenyi *post hoc* test with chi-squared approximation for independent samples (PMCMR v4.1 package; Pohlert 2014) within the R 3.4.0 statistical programming language (R Development Core Team 2016).

Control seedlings, both with and without mesh bags, flushed no epicormic buds (Fig. 1). Destruction of sequential buds, by either scissors (P < 0.001 in all cases) or western spruce budworm larvae (P = 0.029), significantly increased numbers of epicormic buds over the control seedlings (Fig. 1). Seedlings defoliated by larvae had significantly fewer epicormic buds than those defoliated with scissors (P = 0.034 for scissors, and P = 0.026 for scissors+mesh). In most cases, epicormic buds on seedlings with larvae showed feeding damage.



**Figure 1.** Numbers of epicormic buds per two-year-old Douglas-fir seedling. Control seedlings were not defoliated; other treatments had expanding spring buds removed with scissors or by feeding of third- to fifth-instar western spruce budworm larvae. Treatments including "Mesh" had seedlings contained in white, polyester-netting cylinders. Boxplots portray the median (midbar in the box), the 25th and 75th percentiles (box), lowest and highest points within 1.5x the inter-quartile range (lower and upper vertical lines), and outliers (small circles). Large circles show the mean values.

Seedlings defoliated by any means responded with production of new foliage late in the summer. Seedlings with larvae caged on them had significantly fewer flushing epicormic buds than did seedlings that had been defoliated with scissors. Defoliation by insects can have different impacts than defoliation using scissors (Piene and Little 1990); however, in the current experiment, the apparent reduction in epicormic buds in the budworm-defoliated seedlings may have been due to the continued feeding activity of the larvae. It was not possible to distinguish between sequential and epicormic buds once they had been eaten, as individual buds were not tracked in detail in this experiment.

The larval collection sites north of Williams Lake, B.C., experienced 1,539–1,688 mean annual growing degree days, and 174–180 mean frost-free days from 1961–1990 (<a href="http://www.climatewna.com/">http://www.climatewna.com/</a> accessed 2017). Sites near Monte Creek, B.C., where outbreaks have occurred more commonly, have experienced 1,910 mean annual growing degree days and 207 mean frost-free days from 1961–1990 (<a href="http://www.climatewna.com/">http://www.climatewna.com/</a> accessed 2017). Both the number of growing degree days and the number of frost-free days can vary substantially with location and year; in general, a shorter growing season can be expected in the north and at higher elevations, with a greater risk of early fall frosts, than in the south at lower elevations.

These results suggest that northern trees may experience greater growth losses and potentially higher mortality during western spruce budworm outbreaks than might be anticipated further south. Synchrony between larvae and their host trees is a key component of the population dynamics of this insect; both the beginning and end of the phenological window are important to survival and fecundity (Nealis 2012). The availability of high nutritional—quality buds during later instars could improve the survival and fecundity of larvae at the end of the phenological window (Régnière and Nealis 2016). Depending on location and local weather conditions, however, the short growing season in the north could also result in higher insect mortality in some years.

Further work is needed to investigate the interaction between western spruce budworm larvae and epicormic buds at northern latitudes and at higher elevations. Experiments that track individual buds, compare the effects of natural larval feeding to bud removal later in the season, and examine the impacts on mature trees would all improve understanding of defoliator impacts on northern stands. If field populations of mature trees carry less new foliage late in the summer in the north than they do in the south, the impact of a western spruce budworm outbreak could be more severe in the northern part of the insect's range.

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#### NATURAL HISTORY AND OBSERVATIONS

# Archilestes californicus McLachlan (Odonata: Zygoptera: Lestidae):a damselfly new to Canada

#### ROBERT A. CANNINGS1 & RUSSELL V. PYM2

Archilestes californicus McLachlan (California Spreadwing) is a large damselfly native to western North America, ranging from Washington and Idaho south to New Mexico, Arizona and California and, in Mexico, to Sonora and Baja California Sur (Paulson 2011; Westfall and May 2006). This note records the species for the first time in Canada—from three sites in the southern Okanagan Valley, British Columbia (BC; Figure 1).

Russell Pym saw several males and females at a small, shallow, artificial pond at the end of an artificial stream near the entrance to the Liquidity Winery at 4720 Allendale Road, Okanagan Falls, BC (49.32553°N, 119.54993°W). He observed them from 13:00 to 14:00 PDT on 26 September 2016; one male was photographed (Figure 2). From 16:30 to 17:00 PDT the same day, he recorded a female in knee-high grass, three to four metres from the shore of a dugout pond across the road from Walnut Beach Resort, 4200 Lakeshore Drive, Osoyoos, BC (49.01825°N, 119.43580°W). Cattail (*Typha latifolia*) and willows (*Salix* spp.) lined the pond margins.

At the north end of Vaseux Lake the next day, 27 September 2016, Russell photographed a lone male (Figure 3) perched on cattails in a mixed willow swamp and cattail marsh (13:00 to 14:30 PDT). The site was along the boardwalk to the bird blind at 49.30348°N, 119.53696°W.

Archilestes (Stream Spreadwings) is a New World genus of eight species; two are North American, the others live from Mexico to Argentina (Paulson 2009). These damselflies are larger than the related Lestes (Pond Spreadwings) species, which are common and more familiar to Canadian observers.

Archilestes californicus is a large spreadwing (42–60 mm long) with eyes and labrum blue in males. The thorax is metallic brown dorsally, white laterally on the metepisternum and metepimeron, with a brown stripe on the metapleural suture dividing the white areas. The resulting white stripes are good field marks. The pterostigmas are white or tan. The abdomen is brown dorsally, slightly metallic and often with a green tinge; segments 9–10 are pruinose white in males (Figures 2 & 3). Paraprocts are short and parallel, visible from above. Females are coloured as males, but lack pruinosity; the eyes are dull blue to brown; the ovipositor reaches the tip of segment 10.

The flight season is late; in Washington, adults fly from July to November (Paulson 2009). Manolis (2003) records that the breeding season in California is mainly in September and October; this is probably the case in much of its range. *Archilestes californicus* lives along small, slow, often intermittent streams and associated ponds. River backwaters are also inhabited. Paulson (2009, 2013) notes that larvae often swim in open water like little minnows and he believes that waters lacking fish are important to this species. Adults mate and lay eggs where willows and alders line the shore. When not breeding, they often leave the water, flying out into open woodland, fields, and sagebrush grassland (Manolis 2003; Paulson 2009).

Individuals fly out from their perches in waterside shrubs to catch prey and return quickly. When disturbed, they dart into dense vegetation (Kennedy 1915; Manolis 2003).

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Males often perch conspicuously on dead twigs, spreading their wings, defending small territories (Paulson 2009). Pairs oviposit in tandem in willow or alder branches about 0.5 –1.0 cm thick, often up to 3 m above the water. The female inserts a group of six eggs into the cambium, then backs down the stem briefly and repeats the process, laying up to 180 eggs per session. The eggs apparently overwinter before hatching (Kennedy 1915; Manolis 2003; Paulson 2009).

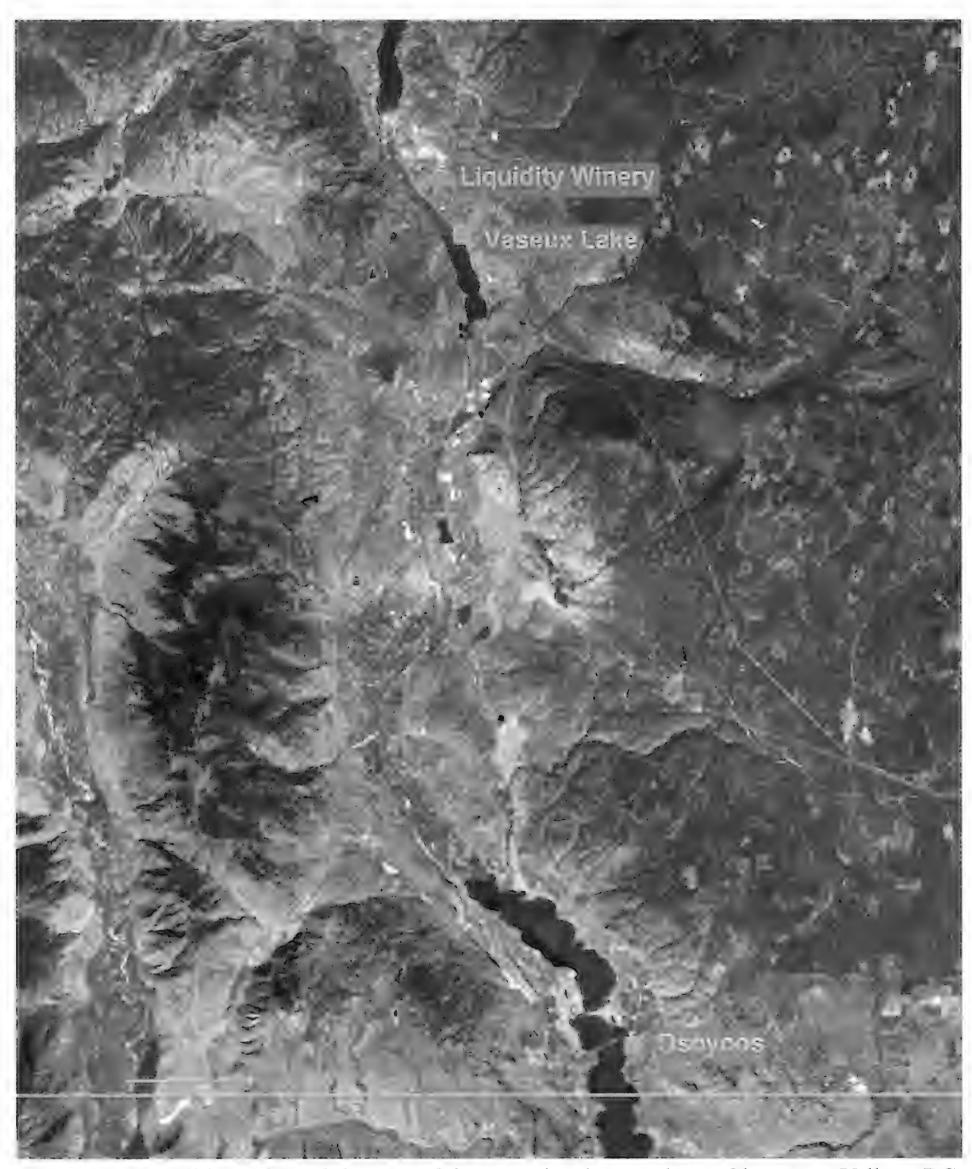
Kennedy (1915) found *Archilestes californicus* abundant at Satus Creek and at other locations in the Yakima Valley of south—central Washington in 1913 and noted that these were the only records north of California at the time. He also corrected an earlier record of *A. grandis* from Yakima that, as Paulson (1970) clarified, should be referred to *A. californicus*. Since then, the species has been recorded at many localities in Oregon and Washington. In Washington, west of the Cascade Mountains, first county records in the *OdonataCentral* website (Abbott 2006–2016) roughly indicate a northern movement: Clark County, 1997; Thurston County, 2009; King County, 2011. Jim Johnson (Abbott 2006–2016; pers. comm.) finds it commonly in Clark County near the mouth of the Columbia River. Dennis Paulson (pers. comm.) says that "it really is moving north. It's common in parts of Seattle now, definitely consolidating its range in this state." The assumption that this is a natural range extension is complicated by the possibility that some of the wetlands involved in Thurston and King counties are artificially constructed wetlands surrounded by planted willows, in which the eggs of *Archilestes* may have been introduced (Paulson 2013).

East of the Cascades, where the species was first reported in Washington, it is recorded in Adams, Benton, Douglas, Grant, Kittitas, Okanogan, Whitman and Yakima counties. There is a broad corridor of counties, from the Columbia River north to the Canadian border, in which the species has been found. Most relevant to the new Canadian records, Jim Johnson found it in the southwest corner of Okanogan County in a pond along Black Creek Canyon (48.07006°N, 120.01917°W) on 1 September 2002 (Abbott 2006–2016). This is 114 km southwest of the Osoyoos, BC, site.

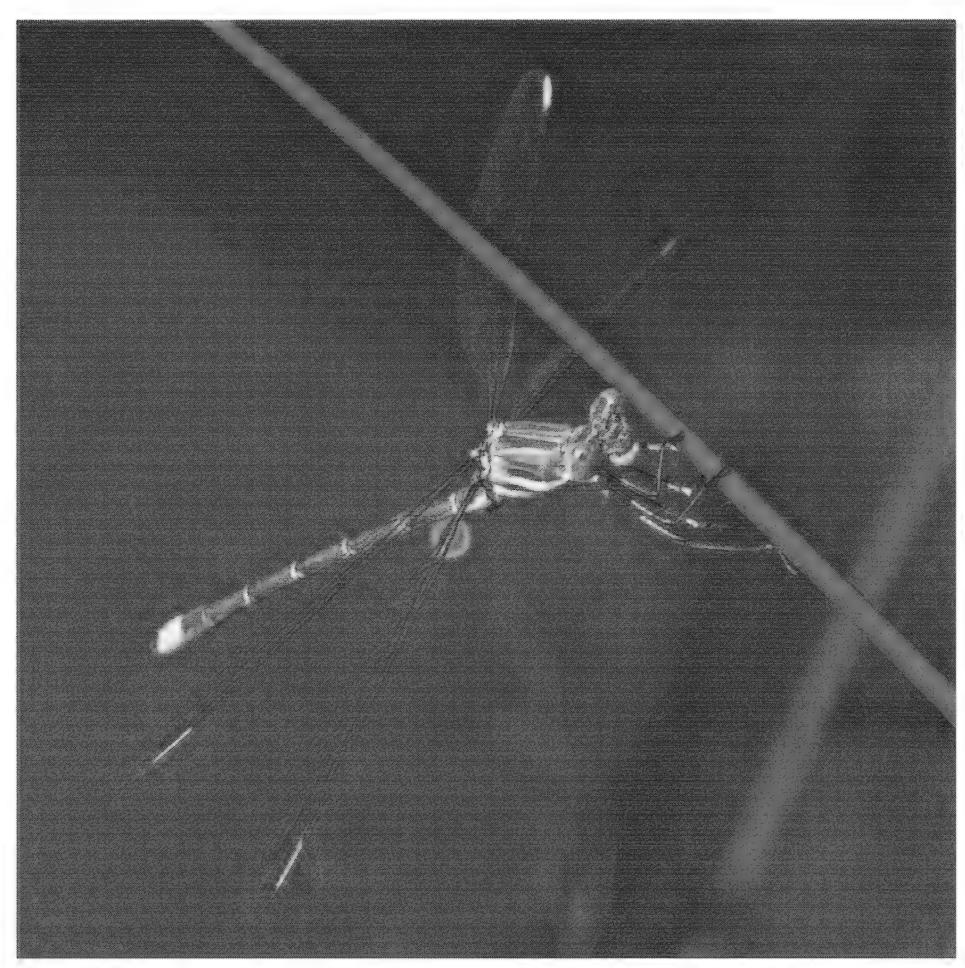
Based on this history, it is not surprising that *A. californicus* has finally appeared in the Okanagan Valley in Canada. The Osoyoos locality is only 2 km north of the United States border. The Vaseux Lake site is 37.2 km north of the Osoyoos site and 2.5 km south of the Okanagan Falls locality. The number of sites reported and the significant distances between them suggest that *Archilestes* probably lives at additional locations in the area and may have been overlooked in the Canadian part of the valley for several years, or at least long enough for it to expand northward more than 40 km from the United States. Further observations will clarify the status of the damselfly in British Columbia and Canada.

Archilestes grandis (Rambur) (Great Spreadwing) is the only other North American species in the genus. It comes no closer to British Columbia than northern California, ranging from California east to New England and extreme southwestern Ontario, and south to Venezuela (Paulson 2009, 2011). It is larger than A. californicus; mature males are darker overall and have more extensive green highlights. The pterostigmas are dark. The pale lateral thoracic stripe is longer and usually yellow rather than white. The paraprocts are divergent under the cerci, and are difficult to see from above (Paulson 2009).

Archilestes grandis has also been on the move—and for a long time. It was first recorded and described from the southwestern states, but its spread to the east and northeast is well documented: it was recorded in Ohio as early as 1931 and, in Canada, at Windsor in 2002 (Pratt and Paiero 2004). Paulson (2011) postulates that this impressive range expansion might have been assisted by the damselfly's tolerance of poor-quality streams, which makes it a successful competitor.



**Figure 1.** Localities of *Archilestes californicus* in the southern Okanagan Valley, BC, September 2016. See text for details. Yellow horizontal line represents the Canada–United States boundary (49° N). Scale line = 5 km.



**Figure 2.** Male *Archilestes californicus* photographed by Russell Pym at Liquidity Winery, Okanagan Falls, BC, 26 September 2016.



**Figure 3.** Male *Archilestes californicus* photographed by Russell Pym at the north end of Vaseux Lake, BC, 27 September 2016.

#### **ACKNOWLEDGEMENTS**

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#### NATURAL HISTORY AND OBSERVATIONS

# Evidence of established brown marmorated stink bug populations in British Columbia, Canada

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**ABSTRACT**— We report four new detections of invasive agricultural pest *Halyomorpha halys* (Stål) (Hempitera: Pentatomidae), the brown marmorated stink bug, in the Lower Mainland and Okanagan Valley regions of British Columbia (BC), Canada, in 2015 and 2016. These finds include two confirmed breeding populations, as well as homeowner collections at the same residence in two consecutive years. Preliminary comparisons of mitochondrial DNA haplotypes from these collections suggest that *H. halys* populations in BC are the result of movement and spread of existing populations in North America, likely from the Pacific Northwest USA.

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), native to Asia, is a globally invasive pest with a broad host-plant range. This species often causes economic losses to tree fruit, berries, vegetables, and ornamental plants (reviewed in Rice *et al.* 2014). The stink bug is also a nuisance pest for homeowners when it seeks indoor overwintering sites in the fall and winter months. Since the detection of new invasive populations, beginning more than 20 years ago, *H. halys* has become broadly established in Europe and North America (reviewed in Haye *et al.* 2015), including most of the continental USA. (see http://www.stopbmsb.org/where-is-bmsb/state-by-state/) and the Canadian provinces of Ontario (Fogain and Graff 2011; Gariepy *et al.* 2014a) and Quebec (Jacques Brodeur, personal communication). In British Columbia (BC), *H. halys* has been intercepted in shipments from Japan, Korea, and China several times since 1993 (Fogain and Graff 2011; Gariepy *et al.* 2014b), but breeding populations were not detected.

From 2015 to 2016, we detected a total of 487 *H. halys* at four different sites in the Lower Mainland and Okanagan Valley regions of BC (Table 1). Evidence of *H. halys* reproduction (eggs and/or nymphs with adults) was found on host plants at one site in the Lower Mainland (Chilliwack Mountain) and another in the Okanagan Valley. At the Chilliwack (Rosedale) site, where the first detection was made in 2015, an increased number of stink bugs returned to the same residence in the fall of 2016, indicating that a breeding population is established in this area. An *H. halys* nymph and two adults were found by two different residents in the Kitsilano neighborhood of Vancouver, BC.

Possible routes of brown marmorated stink bug invasion into BC include natural spread of already-established populations in the Pacific Northwest of the USA (Oregon,

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Table 1
Brown marmorated stink bug (Halyomorpha halys) found in British Columbia in 2015–2016.

Locality (GPS Coordinates) <sup>a</sup>	Collection Dates/ Time period	Initial detection method	Number of specimens and life stage(s)	Location/context of collected H. halys	COI Haplotypes (N individuals) <sup>b</sup>
Chilliwack Mountain) 49°09'34.5"N 121°59'49.2"W	May-Oct 2016	Homeowner find, followed by weekly beat sheet sampling and traps	176 nymphs, 264 adults	Private garden feeding on Asian pear tree (Pyrus pyrifolia) and aggregating on and inside neighboring house. Homeowner reported infestation had been present since 2014.	H1 (18) H3 (25) Hn (8)
Chilliwack (Rosedale) 49°11'04.2"N 121°47'54.1"W	Oct 2015, Sept-Oct 2016	Homeowner find	2 adults (2015) 30 adults (2016)	Exterior walls and roof of house	H1 (4) H3 (2) Hn (1)
Vancouver (Kitsilano) 49°15'50.9"N 123°10'12.3"W and 49°15'49.4"N 123°09'07.8"W	Oct 2016	Homeowner finds	1 nymph, 2 adults	Backyard strawberry plant (1 nymph, 1 adult), and on public transit (1 adult)	<b>H</b> 3
Penticton 49°28'23.1"N 119°35'23.4"W	May, Aug-Oct 2016	Museum collection fieldwork for other taxonomic research; collected with beating tray	4 adults (May 2016), 6 nymphs and 2 adults (Aug–Oct 2016)	Feeding on chokecherry ( <i>Prunus virginiana</i> ) bush near waterway; last adult and nymph of season caught in pheromone-baited Pyramid traps <sup>c</sup> .	H1 (2) H3 (1) Hn (2)

<sup>a</sup> GPS Coordinates are approximate to protect homeowner privacy.

<sup>b</sup> Genbank accession numbers: KF273380 (H1), KF273382 (H3), from Gariepy *et al.* (2014b), KY570297 (Hn).

<sup>c</sup> Baited with Stink Bug Xtra Combo Dead-Inn lures (AgBio, USA).

Washington), as well as accidental human-mediated translocation from Asia, Europe, or other Canadian or American localities. To gather preliminary information on possible invasion routes for this species in BC, we used COI haplotyping, which has previously been used to identify possible sources of H. halys invasions in Canada, the USA, and Europe (e.g., Gariepy et al. 2014b; Xu et al. 2014; Gariepy et al. 2015). We amplified and sequenced a 658-base pair (bp) region of the mitochondrial Cytochrome C oxidase subunit 1 (COI) gene from several specimens collected at each site (Table 1) to identify COI haplotypes (see Gariepy et al. 2014b for primers and methodology). Previous studies concluded that eastern North American H. halys populations originated from a single source population in the Beijing area of China. In contrast, European populations are derived from several Asian source populations, including China, Korea, and other currently unidentified locations (Gariepy et al. 2015). Populations from the western USA (California, Oregon, Washington) include multiple haplotypes, some of which differ from those found in eastern North America (Haye et al. 2015). Our genetic analysis of fieldcollected specimens from BC supports these findings, demonstrating that multiple haplotypes also occur in western Canada. In total, three COI haplotypes were detected among the specimens collected in BC: H1, H3, and a currently undescribed COI haplotype (Hn) (Table 1). COI haplotypes H1 and H3 were described by Gariepy et al. (2014); H1 is the predominant haplotype in eastern North America and some areas of Europe, whereas H3 is known from several regions in Europe, predominantly in Switzerland (Gariepy et al. 2015). Concurrent research in the western USA has employed different gene regions for haplotype analysis (COII and 12S), but comparison of representative datasets demonstrates that COI H1 and H3 haplotypes are already known from Washington and Oregon (Marie Claude Bon and Kim Hoelmer, personal communications). The third COI haplotype (Hn) has not previously been described from samples collected in Asia, Canada, or Europe (Gariepy et al. 2014b; Gariepy et al. 2015). This haplotype may occur in the Pacific Northwest of the USA; however, additional DNA sequencing will be necessary to determine how it corresponds to the haplotypes for the COII and 12S genes that have been analysed for H. halys specimens collected in Washington, Oregon, and California (Marie Claude Bon and Kim Hoelmer, personal communication).

Continuing public outreach will be important for tracking the spread of *H. halys* populations in BC and for gathering specimens to examine population genetics and detect invasion pathways. For example, a citizen discovered the Chilliwack Mountain population following a BC Ministry of Agriculture newspaper advertisement, and the Kitsilano reports were a result of residents seeing news articles reporting on the brown marmorated stink bug. Additionally, just before the submission of this note, two additional citizen reports of individual finds in the Lower Mainland (Langley, BC) and the Interior (Kelowna, BC) were received (Hueppelsheuser and Acheampong, unpublished data). To continue to track the spread and establishment of the brown marmorated stink bug in BC, a citizen science approach is planned, to be complemented by pheromone trapping and grid-based beating sheet sampling of known host plants.

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#### NATURAL HISTORY AND OBSERVATIONS

## An unusual specimen of the subgenus *Lasioglossum* Curtis from British Columbia, Canada (Hymenoptera, Halictidae)

#### CORY S. SHEFFIELD<sup>1</sup> and JENNIFER HERON<sup>2</sup>

The genus Lasioglossum Curtis s. l. (Halictidae) represents one of the largest and most taxonomically difficult groups of bees (Michener 2007; Gibbs 2010a), with at least 1,750 described species (Gibbs et al. 2012). In North America north of Mexico, six subgenera are currently recognized: Dialictus Robertson, Evylaeus Robertson, Hemihalictus Cockerell, Lasioglossum s. str., Sphecodogastra Ashmead, and the introduced Leuchalictus Warncke. Globally, all subgenera have been placed within one of two Lasioglossum "series" (Michener 2007; Gibbs et al. 2012). The Lasioglossum series contains species in which only one (i.e., the third) submarginal crossvein (i.e., 1rs-m) is weakened, and is represented in North America by the subgenera Lasioglossum s. str. and Leuchalictus. All other subgenera in North America normally have two weakened submarginal crossveins and have been placed in the Hemihalictus series. However, weak venation is not always perceptible in males and some female specimens of Lasioglossum s. l., and Ebmer (1969), Michener (2007) and Gibbs et al. (2013) stress the difficulty in placing these problematic specimens within subgenera and even within series using these diagnostic characters.

The problems with wing venation in Lasioglossum are not limited to these difficult cases, as recently summarized by Gibbs (2010b). Hemihalictus, as originally defined by Cockerell (1897), was monotypic, the species L. lustrans (Cockerell) differing from all other non-metallic Hemihalictus series Lasioglossum in having only two submarginal cells; Cockerell (1897) used this character to separate his H. lustrans from all other Halictus Latreille. However, Gibbs (2010b) and Gibbs et al. (2013) showed that a small proportion of specimens of L. lustrans have three submarginal cells. Similarly, Dialictus was originally defined (Robertson 1902a) as monotypic and included one metallic species with two submarginal cells, L. anomalum (Robertson); Chloralictus was used to distinguish metallic species with three submarginal cells with two weakened submarginal crossveins (Robertson 1902b). Gibbs (2010b) indicated that L. anomalum is also known to have individuals with three submarginal cells. Therefore, wing venation alone is not always reliable for species- or subgenus-level identification in Lasioglossum s. l. In fact, Stephen et al. (1969) felt that these differences were so minimal that they considered many of the taxa currently recognized as subgenera of Lasioglossum to be subgenera of Halictus.

Both *Hemihalictus* and *Dialictus* are now defined much more broadly than these historic usages. Based on phylogenetic data, Gibbs *et al.* (2013) placed many species of *Evylaeus* (i.e., the non-metallic carinaless species, or non-metallic *Dialictus*) into the subgenus *Hemihalictus*; thus, the subgenus is no longer considered monotypic (as per Michener 2007). Mitchell (1960) was the first to define *Dialictus* (at genus level) as all metallic Halictinae with two submarginal cells or two weakened submarginal crossveins. Michener *et al.* (1994) were among the first to consider these as subgenera of *Lasioglossum*. To this date, two submarginal-celled forms are known from only the

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Hemihalictus series of Lasioglossum, specifically in the subgenera Hemihalictus and Dialictus.

Our objective here is to describe an aberrant specimen of Lasioglossum s. str. from British Columbia, Canada; it therefore represents the first documented case of a twosubmarginal celled individual within the Lasioglossum series. As part of ongoing work on bee diversity and taxonomy in Canada, specimens were collected throughout the Western Interior Basin Ecozone (=Southern Interior Ecoprovince) of British Columbia. This area is considered the most bee species-rich in the country, with more than 50% of Canada's bee species known from this relatively small area (5.7 million ha), over 1/3 of which have not been recorded elsewhere in the country (Sheffield et al. 2014; Heron and Sheffield 2016). Among the specimens collected from this area between 2009 and 2016, one male specimen of Lasioglossum collected on Mt. Kobau, South Okanagan Grasslands Protected Area, near Osoyoos [49.106, -119.651, 08 Aug 2014, Col. C. Sheffield] was morphologically unique in being a non-metallic Lasioglossum s. l. with two submarginal cells (Figure 1), thus superficially resembling L. lustrans of eastern North America, although with antenna resembling Lasioglossum s. str. (McGinley 1986). Since the "two submarginal cell" condition of L. lustrans and L. anomalum is not always consistent (Gibbs 2010b) and because no Halictinae with two-submarginal cells in the forewing have been previously recorded from western North America (Stephen et al. 1969), including western Canada (Gibbs 2010b), DNA barcoding was used to compare sequences from the specimen (BOLD Sample ID CCDB-20945 F01) to other specimens from western Canada (following methods of Sheffield et al. 2009, 2017). Despite examining thousands of specimens from the Western Interior Basin, including from the Mt. Kobau area of British Columbia, no additional specimens of Lasioglossum with two submarginal cells could be found. The specimen in question (Figure 1) was identified as a member of the subgenus Lasioglossum s. str. due to the characteristic basal antennal structure (Figure 2; proportion of length of flagellomere 1 to 2), and tentatively as L. (Lasioglossum) sisymbrii (Cockerell) largely due to the characteristic pale, translucent tegulae (Figure 1) and by dissection and examination of the genitalia (Figure 3), including sternum 8 (Figure 4) (McGinley 1986); this identification was supported through comparison of COI sequences, matching identically with material in BOLD identified as this species (see Sheffield et al. 2017). Although both sexes of this species typically have a complete basal hair band on tergum 1 (McGinley 1986), this was probably worn on our specimen. Our specimen also differed in having rather pale tarsi (Figure 1)—not concolorous with the tibiae (McGinley 1986).

A puzzling feature of our male specimen is the 12-segmented antennae (i.e., 10 flagellomeres; Figure 2)—the normal condition for female bees. Males of most bees have 13-segmented antennae, although males of *Cherogas* (Halictidae: Augochlorini) and a few other genera are 12-segmented (Michener 2007; Engel 2010). One possibility is that the specimen is a gynandromorph, although this condition has not been reported previously for this species (see Wcislo *et al.* 2004; Michez *et al.* 2009; Hinojosa–Díaz *et al.* 2012). If the specimen is indeed a gynandromorph, this condition seems restricted only to the antennae, and the specimen otherwise resembles a male. Wcislo *et al.* (2004), Michez *et al.* (2009), and Hinojosa–Díaz *et al.* (2012) have listed bee species known as gynandromorhps, the most recent study indicating that it has only been observed in 21 species of Halictidae, eight of which are *Lasioglossum s. l.* The other possibility is that this condition represents a development anomaly with this specimen.

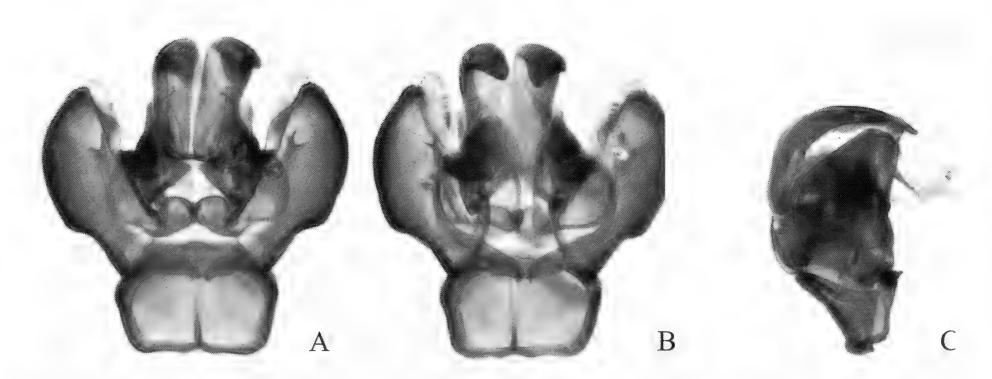
This specimen of *L. sisymbrii* becomes the first account of a member of the *Lasioglossum* series having two submarginal cells, and perhaps the first reported case of gynandromorphy in a North American *Lasioglossum*. As with the other published works cited above, we feel that documenting such anomalous specimens is important to account for the variation that exists within species and, as discussed above, variable wing venation has had an important impact on nomenclatural history for sweat bees in the past.



**Figure 1.** Lateral habitus of male *Lasioglossum sisymbrii* (Cockerell), with two submarginal cells in each forewing (numbered for the left forewing).



**Figure 2.** Face of male *Lasioglossum sisymbrii* (Cockerell) showing the 12-segmented antennae (i.e., 10 flagellomeres; the normal condition in males is 11 flagellomeres).



**Figure 3.** Genitalia *Lasioglossum sisymbrii* (Cockerell) – A) dorsal, B) ventral, and C) lateral views.

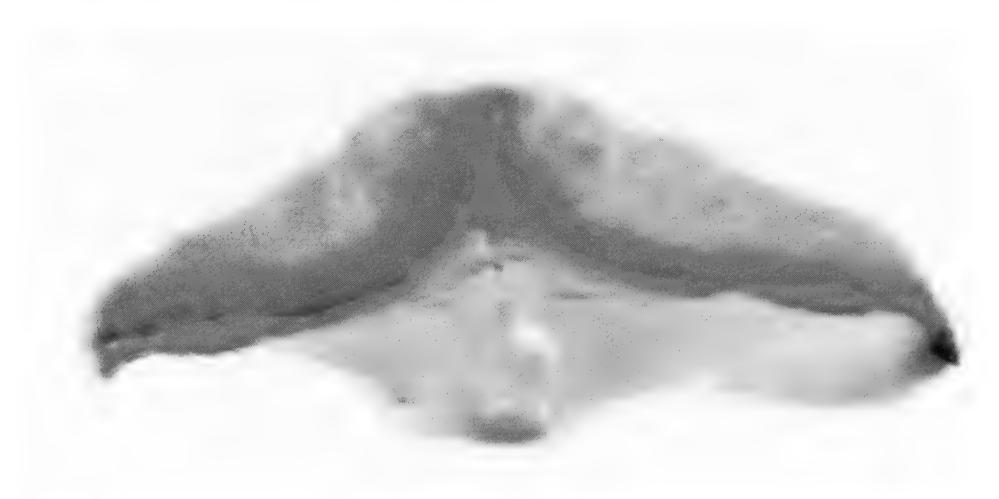


Figure 4. Sternum 8 of Lasioglossum sisymbrii (Cockerell).

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#### NATURAL HISTORY AND OBSERVATIONS

# First identifications of aphid and diamondback moth populations on wasabi in British Columbia

#### JESSE L. MACDONALD<sup>1,2</sup>, ERIC MAW<sup>3</sup>, and PEGGY CLARKE<sup>4</sup>

#### **ABSTRACT**

Wasabi is a highly valued crop in the Pacific Northwest where commercial production is increasing. To date, little attention has been paid to its invertebrate pests. Two wasabi polyhouses in Agassiz, BC, were monitored for insect pests for 15 months. *Pemphigus populitransversus* Riley (poplar petiole gall aphid) recurred annually in winter months on roots throughout the polyhouses. *Lipaphis pseudobrassicae* Davis (turnip aphid) infested the leaves of a large number of plants. *Myzus persicae* Sulzer (green peach aphid) and *Macrosiphum euphorbiae* Thomas (potato aphid) were noted in very low numbers. *Plutella xylostella* Linnaeus (diamondback moth) caused shot-hole damage of the leaves. Further investigation into the role of insects as vectors and their role in pathogen pathways on this unique crop is needed.

**Key words:** wasabi, *Pemphigus populitransversus*, *Lipaphis pseudobrassicae*, *Plutella xylostella* 

#### INTRODUCTION

Wasabi (Wasabia japonica (Miq.) Matsumura) (Brassicaceae) is native to Japan, where it grows in shaded stream environments (Adachi 1987). It is currently cultivated in Asia, Australasia, and North America for its valuable rhizome, which is used as a freshlyground condiment eaten with traditional Japanese meals (Hodge 1974; Chadwick et al. 1993). It can fetch US\$150-300/kg on the international market. Although the rhizome is the primary plant part for culinary use, the leaves can also be used to flavour soups or salads (Chadwick et al. 1993). In B.C., there is an estimated 5-10 acres of commercial wasabi in production using hydroponic or similar systems in polyethylene tunnels (polyhouses) or traditional glass greenhouses. Plants are typically grown in river rock substrate, as plants grown in soil are thought to produce an inferior quality rhizome (Chadwick 1993; Sultana et al. 2003). It takes 12 - 18 months before plants are of marketable quality, and due to the humid growing environment and propagation from axillary shoots, disease issues are the most common reason for crop loss in British Columbia (Rodríguez & Punja 2009; Punja et al. 2017; MacDonald & Punja 2017). To date, little research has been directed toward arthropod pests, and all reports are anecdotal. We report the first occurrence of insect pests on wasabi in North America at a research planting in Agassiz, British Columbia.

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#### **MATERIALS AND METHODS**

Two polyhouses, each planted with 500 tissue-cultured (cv. Daruma) and 500 auxiliary-shoot propagated (cv. Mazuma) wasabi plantlets, were established in January 2015 at the Agriculture and Agri-Food Canada (AAFC) Agassiz Research and Development Centre in Agassiz, BC. Prior to transplant into the polyhouses, the plants were maintained in a production greenhouse for one month. A commercial nutrient growing system was used, with overhead misters fertigating at regular intervals or when triggered by a photosensor. Plants were grown in ~20 cm of 2-3 cm diameter river-rock. From April to October, 70% shade cloth covered the polyhouses to reduce direct exposure to UV radiation. Weekly or bi-weekly inspections were conducted by trained staff to identify pests and for treatment recommendations. Aphid populations were treated with imidacloprid. Two releases of *Diadegma insulare* Cresson parasitoids were conducted weekly to manage feeding diamondback moth larvae, followed by treatment with flubendiamide.

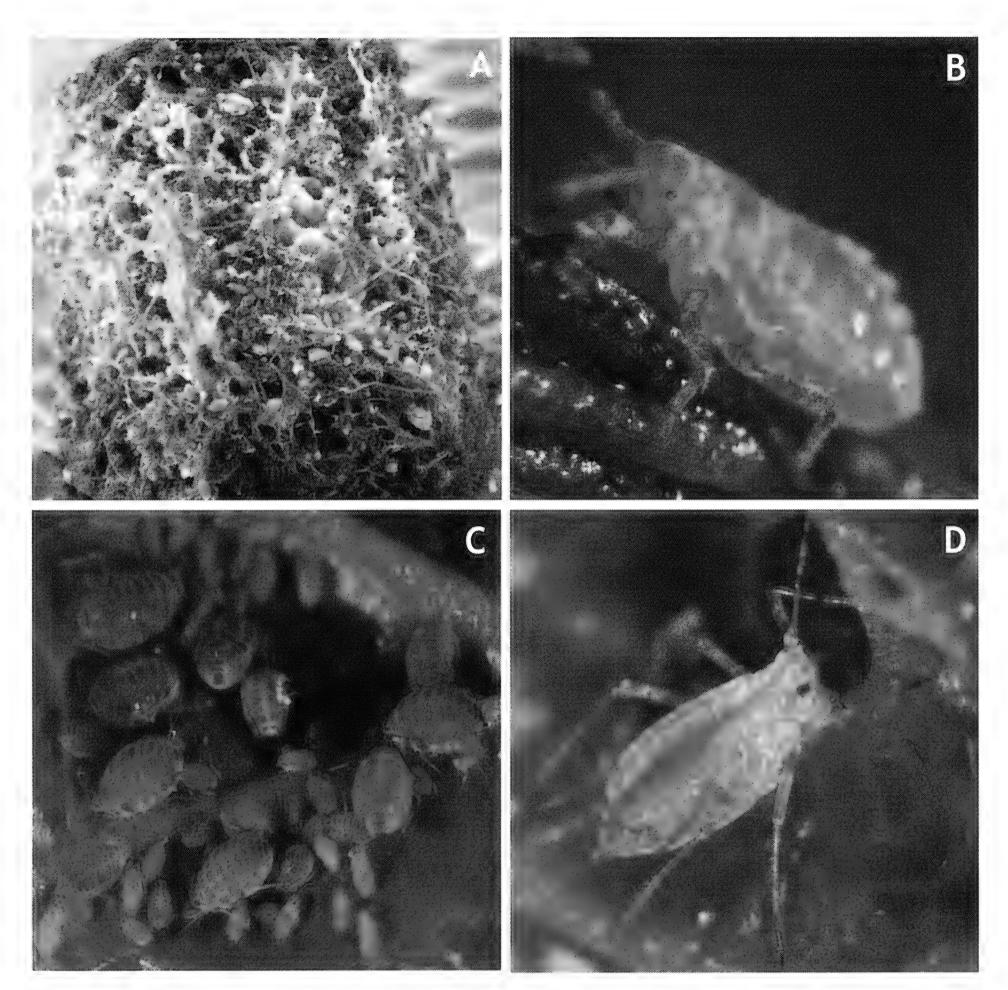
Leaves, petioles, and roots were visually inspected at random throughout each planting. Approximately five infested plants with representative pest populations from either leaves and petioles or roots were selected for each sample. A soft paintbrush was used to gently brush specimens off of plant material into vials of 95% ethanol (EtOH) for identification. Aphids were identified by morphological determination or by sequencing mitochondrial cytochrome C oxidase, subunit 1 ("DNA barcoding"). Adult diamondback moths were identified by morphology under a binocular microscope. Assessments were carried out until harvest, after 15 months.

#### **RESULTS AND DISCUSSION**

Poplar petiole gall aphids. In January 2015, *W. japonica* 'Daruma' plantlets grown in soil-less plugs were uprooted for transplant into polyhouses and a heavy root aphid infestation was noted. Aphids were present throughout the crop and each root system, and a characteristic white waxy secretion was visible (Fig. 1A). No alates were present. The following January to March (2016) on the same crop of Daruma and a neighbouring crop of Mazuma, identical root aphid populations were again found. In both cases treatment with imidacloprid appeared to provide control. All populations were identified as *Pemphigus populitransversus* Riley by sequence matching to specimens collected from galls on *Populus deltoides* (Fig. 1A, B).

Root aphids have been implicated as pests of wasabi historically (Miles & Chadwick 2008; Chadwick et al. 1993) but identified only once, in New Zealand, as P. bursarius Linnaeus (Douglas & Follett 1992); that population was difficult to control. Pemphigus populitransversus is known to alternate hosts between roots of various Brassicaceae, sometimes as a significant pest (for example Chen et al. 2009), persisting by parthenogenesis, and a sexual stage on Populus spp. trees, where they overwinter as eggs and form galls on the petioles of the leaves the following spring (Jones & Gillette 1918). Aphid damage to roots and rhizomes may be an important pathway for pathogens such as Pectobacterium carotovorum subsp. carotovorum, which has been found to cause vascular blackening of the rhizome after entry through small wounds (Rodríguez & Punja 2009). This is the first published report of P. populitransversus in B.C. that the authors are aware of, although there are specimens of unidentified Pemphigus species from wasabi collected in Aldergrove and Langley in 1997 and 1998 in the Canadian National Collection of Insects, Arachnids and Nematodes.

**Turnip aphids.** In spring of 2016, aphids were found predominantly on leaves of one-quarter of the affected polyhouse and identified as *Lipaphis pseudobrassicae* Davis (n = 62 specimens). *Macrosiphum euphorbiae* Thomas (n = 2) and *Myzus persicae* Sulzer (n = 1) were also present in the sample.



**Figure 1.** A) Parthenogenic *P. populitransversus* population with waxy exudate in the root mass of a *W. japonica* plant grown in a plug-tray. B) Apterous *P. populitransversus* with proboscis in *W. japonica* root. C) *L. pseudobrassicae* colony consisting of different instars on a *W. japonica* leaf. D) *M. euphorbiae* aptera with proboscis in a *W. japonica* leaf. Photos by J.L. MacDonald, with permission © Her Majesty the Queen in Right of Canada as represented by the Minister of Agriculture and Agri-Food 2016.

The most serious issue associated with aphids on wasabi is their ability to transmit viruses (Douglas & Follett 1992). Wasabi is susceptible to tobacco mosaic virus (TMV), turnip mosaic virus (TuMV), and cucumber mosaic virus (CMV) (Chadwick *et al.* 1993; Wilson 1998), and although problematic elsewhere, no viruses have been identified on wasabi in B.C. Should these diseases be reported locally, *L. pseudobrassicae* should be assessed as a potential vector of TuMV and CMV (Chan *et al.* 1991).

**Diamondback moth.** In June 2015, a heavy infestation of diamondback moth, *Plutella xylostella* Linnaeus, and associated 'shothole' damage on leaves was found. Adults were prevalent and flew as plants were disturbed.

Diamondback moth is the most destructive pest of Brassica crops worldwide. It has been reported on wasabi crops in Japan (Hodge 1974; Adachi 1987; Chadwick *et al.* 1993; Miles & Chadwick 2008). Due to successful management of the infestation with flubendiamide, it is unclear whether *D. insulare* releases were effective. Interestingly, a single mobile parasitoid adult was photographed almost 10 months later in a

neighbouring polyhouse which had no previous biocontrol releases, suggesting the population persisted.

This first survey of insect pests of commercial wasabi production suggests that there is considerable potential for economic damage. Currently, no insecticides are registered in the United States for use on wasabi and only one synthetic insecticide is registered in Canada (permethrin). Although there are a number of biopesticides available in Canada, these may not be sufficient if the aphids are vectors for viruses. Investigations into the relationship between aphids and pathogens (such as *P. carotovorum*), or as vectors of viruses, may generate interest in an integrated management approach, as well as the registration of additional control products for resistance management for use in commercial wasabi crops.

#### **ACKNOWLEDGEMENTS**

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# Proceedings of the Pollination: Science and Stewardship Symposium

## University of British Columbia - Okanagan Campus, Kelowna, British Columbia, March 20, 2017

#### INTRODUCTION

Jennifer Heron<sup>1</sup> and Cory S. Sheffield<sup>2</sup>

Assessing the threats to and the conservation status of pollinators is emerging as one of the greatest challenges facing conservation practitioners today. The diversity of pollinator taxa and their cumulative contributions to natural ecosystem health and human well-being involve complex, albeit often poorly understood, relationships. Increased concern about the plight of pollinators has resulted in increased funding for education and research on these topics, which is strengthening science-based policy and increased public awareness. A symposium was held on March 20, 2017, at the University of British Columbia, Okanagan Campus, in Kelowna, on pollinator science and stewardship. The symposium brought together eight speakers who discussed topics related to pollinator conservation, providing examples and case studies of conservation assessment, public engagement, pollinator policy, and ideas regarding how to address the challenges that are faced by pollinators and pollinator-stewardship practitioners. The symposium also facilitated connections that enable lands managers, owners, stewards and conservation practitioners to take this information and apply it to their own conservation practices.

Support for the symposium was provided by the federal Habitat Stewardship Program for the Prevention of Species At Risk, the B.C. Ministry of Environment and Climate Change Strategy, the Royal Saskatchewan Museum, the British Columbia Conservation Foundation and the Entomological Society of British Columbia.

#### The buzz on Yukon bees

Syd Cannings, Environment and Climate Change Canada, Canadian Wildlife Service, Whitehorse, YT Y1A 5X7

Amid the growing concern for the fate of bees, I have begun several studies on bees in northern British Columbia and the Yukon, collaborating with Paul Williams at the Natural History Museum (UK) and Cory Sheffield (Royal Saskatchewan Museum). Over the past six years, these studies have revolved around focused collecting with nets and traps in the various ecosystems of the north.

In general, bumblebee species that have declined dramatically in the south (e.g., the Western Bumblebee [Bombus occidentalis occidentalis] and Yellow-banded Bumblebee [Bombus terricola]) are still common in the southern Yukon. However, the Gypsy Cuckoo Bumblebee (Bombus bohemicus; assessed Endangered in Canada by the Committee on the Status of Endangered Wildlife in Canada [COSEWIC]) seems to be much sparser than it was in the 1980s. We have found this species in two localities: Stewart Crossing in 2014 and Kluane in 2016. These are the only detections of this species in North America in the past five years.

Mitochondrial DNA analysis of some of the bumblebees we've collected has revealed a new species of subarctic bumblebee in the subgenus *Alpinobombus*, now named *Bombus kluanensis*. Even though we now have a better idea of the status of most northern bees, we do not have good data on ongoing trends. To tackle that issue, we are

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planning to institute a repeatable monitoring plan for bumblebees in the Yukon and northern B.C., modeled after the North American Breeding Bird Survey.

#### Honeybees and honeybee health

Rob W. Currie, University of Manitoba, Faculty of Agricultural and Food Sciences, Department of Entomology, Winnipeg, MB R3T 2N2

Honey bees (*Apis melanifera*) have been experiencing high levels of colony loss on a regular basis over the past decade. While speculation originally centered on the idea that there was a single mysterious cause, we now know that multiple stressors are interacting, sometimes in unpredictable ways to cause problems for this critically important crop pollinator. Exciting progress is being made on high- and low tech-solutions to help mitigate these losses, and some of these research innovations include using molecular and proteomic markers, as well as conventional approaches to breed bees for resistance to parasites and pathogens. Managing viruses through more effective management of their primary vector, the *varroa* mite, and using RNAi to control viruses also can be effective in helping beekeepers mitigate losses from some of the more critical stressors in the system.

#### COSEWIC and the General Status of Species in Canada

David F. Fraser, British Columbia Ministry of Environment and Climate Change Strategy, Species Conservation Science Unit, Victoria, B.C. V8W 1M8

The status of species at risk in Canada is assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). COSEWIC recommends species in the atrisk category to the federal Minister of Environment and Climate Change for listing under the federal Species At Risk Act (SARA). To date, there have been 976 wildlife species assessed by COSEWIC, and 521 of these are listed on Schedule 1 of SARA. The Program on the General Status of Species in Canada provides overview of the status of biodiversity in Canada every five years and bears no legal implication. In 2013, this program moved to using the same assessment system as used by NatureServe and the B.C. Conservation Data Centre. The latest General Status report, covering the 2000–2015 timespan, assessed 29,848 species. The COSEWIC assessment process requires extensive time and resources, and prioritizing which species to recommend for assessment is a challenging task. Results from the General Status are one of the inputs that helps guide the determination of which species are priorities. In addition, other factors, such as the percent of the species range in Canada, the species global status, and the pattern of decline, is used by COSEWIC to modify the priority score a species is given. A thorough understanding of both the General Status and COSEWIC processes is important for prioritizing species recommended for status assessment.

#### **Butterfly conservation in Canada: threats and challenges**

Jennifer M. Heron, British Columbia Ministry of Environment and Climate Change Strategy, Species Conservation Science Unit, Vancouver, B.C. V3R 1E1

Butterflies are a well-known and well-studied group of pollinators. Approximately 275 butterfly species are known to occur in Canada, although only 21 have been assessed nationally by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). There are numerous challenges to assessing the lesser known and poorly documented butterfly species, particularly when the host plant(s) are unknown, the threats are unclear, and the species' habitat and associated plant communities are undescribed. Museum collections are important sources for historical information and, at one time, butterfly specimens were more widely collected and deposited at museums. However, historical collection data are often biased, not databased, or incorrectly identified. In the past few decades, butterfly surveys have moved away from specimen collection and museum deposition, and instead focus on visual surveys or photographic evidence—a method that is good for conserving populations but has other drawbacks.

Using examples from the south Okanagan, this talk will provide an overview of the challenges to assessing butterflies, how candidate species are recommended for COSEWIC assessment, challenges to assessing the lesser known species, and ways conservation practitioners can include butterflies in land management decisions and planning.

## Border Free Bees: artists linking science and communities for pollinator conservation

Nancy Holmes and Fionncara MacEoin, The University of British Columbia, Okanagan Campus, Faculty of Creative and Critical Studies, Kelowna, B.C. V1V 1V7

Border Free Bees (BFB) is a Social Sciences and Humanities Research Council (federal) funded provincial initiative in which artists lead community engagement projects to enhance awareness and inspire action around pollinator conservation. Along with several projects in the Lower Mainland, BFB has two major projects underway in Kelowna—The Public Art Pollinator Pasture and the Kelowna Nectar Trail. Designed to address the decline in native habitat, BFB is an ambitious and creative pollinator-focused arts-based research initiative, headed by Dr. Cameron Cartiere, Associate Professor at Emily Carr University of Art + Design (ECUAD) and Nancy Holmes, Associate Professor in Creative Studies at The University of British Columbia, Okanagan (UBCO). The research project's mission includes raising awareness of the plight of wild pollinators, particularly bees, and transforming underused urban sites into aesthetically pleasing and scientifically viable habitats. Border Free Bees uses public art and design methodologies to empower communities to actively engage in these restoration initiatives and equips individuals and communities with the knowledge and tools to take stewardship of such public projects.

#### From personal to planetary: making an impact on pollination at different scale

Hien T. Ngo, IPBES Secretariat, Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES), UN Campus, Platz der Vereinten Nationen 1, D-53113, Bonn, Germany

Research and initiatives that focus on pollination have impacts on different scales. With the International Pollinator Initiative (UN-FAO), local researchers worked with small-scale farmers using a common method to examine pollination deficits. This was repeated in multiple countries in multiple regions around the world, resulting in a scaled-up key finding regarding the role of wild pollinators in agroecosystems. Recently, the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) completed their Summary for Policymakers and assessment report on Pollinators, Pollination and Food Production. These key findings, which included policy options, were adopted (Decision XIII/15) at the Convention on Biological Diversity Thirteenth Conference of the Parties (COP-13). Furthermore, these key findings have already had an impact on many national pollinator strategies and were the basis of a new multinational initiative, the Coalition of the Willing on Pollinators.

#### Pesticides and pollinators: evidence, controversy and policy

Nigel Raine, University of Guelph, School of Environmental Sciences, Rebanks Family Chair in Pollinator Conservation, Guelph, ON N1G 2W1

Recent concern over global pollinator declines has led to considerable research on pesticide impacts. Here, we report on a series of recent studies that examine the extent to which field-realistic insecticide exposure can lead to significant sublethal impacts on individual bumblebee behaviour (e.g., reduced queen colony founding success and impaired worker learning and foraging), colony function (e.g., effects on growth rates and forager recruitment), and the critical ecosystem services they provide to crops and wild plants. Taken together, these effects could have widespread implications for the stability of wild pollinator populations, sustainable production of pollinator-limited

crops, and maintaining wild-plant biodiversity. Considering these studies that report insecticide impacts on non-Apis (honey) bees into the wider context, particularly alongside divergent results from honey bee field trials, has important potential ramifications for pesticide-use policies.

## Integrated wild pollinator management: putting wild bees to work for crop and wildflower pollination

Cory S. Sheffield, Royal Saskatchewan Museum, Regina, SK S4P 2V7

Bees, unlike many other groups of pollinating insects, are Central Place Foragers, foraging for floral resources in areas surrounding their nest, the radius being approximately equal to the maximum flight distance of the individual species (larger bees typically flying further). For a nesting bee, being restricted to this area has implications for both pollination and conservation, because this landscape must provide ample nectar and pollen and, for some species, nesting materials; areas lacking all the requirements will be abandoned and, over the long term, will lose bee populations. Canada has more than 850 wild bee species, and a large proportion of these are generalist pollen users and visit (thus pollinate) many of our crops. Many of these same species also visit non-crop plants, so provide valuable ecological services to the natural and semi-natural communities surrounding crop lands. Central Place Foraging, body size, flight range, and floral resource availability all have to be considered when considering the use of wild bees for crop pollination and in maintaining populations for pollination in non-crop habitats. These factors, along with life history characteristics of bees, will be discussed in the context of pollination and management of wild bees.

#### **Pollinator Partnership Canada**

Lora Morandin, Pollinator Partnership Canada, Victoria, B.C.

Pollinator Partnership Canada (P2C) is the first international expansion of Pollinator Partnership (P2), which is the largest non-profit organization dedicated solely to the preservation of pollinators and their ecosystems. Pollinator Partnership and P2C work to conserve pollinators through research, policy, outreach and education, collaboration, and habitat creation. Pollinators are directly responsible for providing approximately one-third of the food we eat and are essential to natural ecosystems. Yet, both managed and wild pollinators are facing numerous pressures and population declines due to habitat loss, pest and diseases, invasive species, climate change, and exposure to pesticides. In Canada, P2C has created a national planting guide for honey bee forage in association with the Bee Health Roundtable, created 14 new ecoregional pollinator planting guides with native plant lists, and reviewed Canadian bee habitat programs. We are beginning new programs to promote monarch conservation through research and habitat creation and are launching local networks to facilitate education and collaborative action.

#### **Presentation Abstracts**

# Entomological Society of British Columbia Annual General Meeting Student Union Building, University of the Fraser Valley, Abbotsford, BC October 13, 2017

Yeast enhances the attraction of yellowjackets to dried fruit and fruit powder

Tamara Babcock<sup>1</sup>, R. Gries<sup>1</sup>, L. Palmero<sup>1</sup>, G. Gries<sup>1</sup>, J. Borden<sup>2</sup>, A. Mattiacci<sup>3</sup>, M. Masciocchi<sup>3</sup>, J. Corley<sup>3</sup>, <sup>1</sup>Simon Fraser University, <sup>2</sup>Scotts Canada, <sup>3</sup>GEPI-INTA

There is need for the development of a better trap bait which can effectively trap pestiferous yellowjacket species. We field tested dried fruit and fruit powder baits with and without yeast, and found that the addition of yeast improved the attractiveness of fruit baits to yellowjackets by up to 50-fold.

#### Toxin diversity and specificity in a Drosophila defensive symbiosis

Matt Ballinger and Steve Perlman, Department of Biology, University of Victoria Ribosome-inactivating proteins (RIPs) have been implicated in Spiroplasma symbiont-mediated defense of Drosophila against parasitic nematodes. We test the activity of these toxins against parasitoid wasps, implicating them in protection against endo-but not ectoparasitoids, and examine the role of diverse natural enemies of insects in driving the evolution of bacterial toxin repertoires.

## Population state-dependent invasion potential of the mountain pine beetle in Alberta

Jordan Lewis Burke, Richard Hamelin, Allan Carroll, Dept. of Forest and Conservation Sciences, Faculty of Forestry, University of British Columbia

The mountain pine beetle [MPB] has invaded novel pine habitat in Alberta, and has transitioned from the native species, lodgepole pine, into the novel boreal species, jack pine. While evidence currently supports the prediction that MPB will have an advantage in jack pine during epidemic population phases, the fate of low-density endemic populations is unclear. Here, I present two studies conducted over the last two years, which surveyed trees typically selected by endemic MPB for competitor dynamics in the field, and compared MPB symbiont growth characteristics under a range of conditions and compared these to an antagonistic competitor in the lab. Results demonstrate a clear disadvantage for endemic MPB in jack pine compared to lodgepole. MPB, and potentially other eruptive bark beetles, are likely to exhibit population state-dependent invasion potential, where epidemic behavior may lead to success while endemic behaviors may lead to failure to establish in novel systems with no coevolutionary history. These results support continued effort by forest managers in Alberta to prevent MPB populations from breaching epidemic thresholds, as their populations at low-density are unlikely to be stable, and unable to persist long-term.

## Creating a DNA biomarker to identify dengue refractory and susceptible Aedes aegypti

Heather Coatsworth, Clara Ocampo, Carl Lowenberger, Simon Fraser University
Dengue viruses transmitted by Aedes aegypti infect 50-100 million people each year. In
Colombia, 30% of feral Ae. aegypti are dengue refractory. We conducted a genome-wide
association study comparing susceptible and refractory mosquitoes. Variants were

investigated for possible relevance to the phenotypes. Secondary validation of these variants is currently underway.

## Cutworm Killer: an Okanagan Beauveria bassiana isolate shows promise for climbing cutworm control in vineyards

Naomi DeLury and Tom Lowery, AAFC-SuRDC

Climbing cutworms (Lepidoptera: Noctuidae) are a major pest of grapevines in the Okanagan and Similkameen valleys of BC, attacking grape buds early in the spring when temperatures are low. We compare the efficacy of a local field-collected isolate of *Beauveria* and commercial strains against local and introduced cutworm species.

#### Mixed pathogen interactions: how does host nutrition modulate disease?

Pauline S. Deschodt, Olivia J. H. Walker, Alana K. Breitkreutz and Jenny S. Cory, Department of Biological Sciences, Simon Fraser University

Individual hosts are commonly challenged by multiple pathogen species. Yet, studies on insect-pathogen interactions mainly focus on interactions between a single host and a single pathogen. Two (or more) pathogens co-infecting a host may compete directly (interference) or indirectly, for resources or via the host immune system. These competitive interactions could increase or decrease host mortality, or result in no change, as well as alter the transmission of disease within the population. In insects, increased dietary protein can increase survival, to pathogens such as baculoviruses and bacteria, even when nutrition is altered post-infection. However, the role of nutrition in mixed pathogen infections is not known, but is likely to relate to the relative cost of resistance to different pathogen groups. Using the cabbage looper, Trichoplusia ni, its nucleopolyhedrovirus (TnSNPV) and the entomopathogenic fungus, Beauveria bassiana, we asked whether host nutrition could alter the outcome of a mixed infection. We challenged T. ni larvae with either a single pathogen species or two simultaneously; then reared the larvae on an artificial diet differing in levels of two major macronutrients, protein and digestible carbohydrate (quality) or the total amount of these two macronutrients (quantity). The results suggest that the virus and fungus respond differently to host nutritional intake, especially on different ratios of protein and carbohydrate. As expected, poor quantity diet exacerbates the negative effect of pathogen on host survival. Moreover, in co-infection, the effect of diet composition on host mortality is greater at lower pathogen doses. These results indicate that diet could be an important modulator of mixed infections.

#### Within-individual repeatability of behavioural activity levels of the parasitoid Pachycrepoideus vindemmiae

Wendy Fleming, University of Victoria

A model system for tracking parasitoid behavioural activity levels and connecting them to biological control performance was developed using the pupal parasitoid *Pachycrepoideus vindemmiae*. Three aspects of activity levels were studied: i) circadian patterns; ii) links to sex and body size; and iii) within-individual repeatability ("personality").

#### **How to Train your Parasitoid (in Sawdust)**

Jessica Y.W. Leung<sup>1</sup> and Paul K. Abram<sup>2</sup>, <sup>1</sup>Simon Fraser University, <sup>2</sup>Agriculture & Agri-Food Canada

In a proof-of-concept study, we show that the parasitoid *Pachycrepoideus vindemmiae*, a candidate biological control for the berry pest *Drosophila suzukii*, can be retained for longer in a realistic substrate where hosts are usually present (sawdust mulch) when it has been "trained" to associate the substrate with *D. suzukii* pupae.

#### Synthetic Aphid Honeydew Volatiles Attract Mosquitoes (Diptera: Culicidae)

Dan A.H. Peach, N. Young, R. Gries, S. Kumar, G. Gries, Simon Fraser University
Adult mosquitoes exploit a variety of plant sugar sources. Plant-derived semiochemicals guide mosquitoes to inflorescences and fruit, but the cues that attract mosquitoes to other sources remain largely speculative. Drawing on literature reports of aphid honeydew volatiles, we tested the attraction of synthetic honeydew volatile blends to Aedes aegypti mosquitoes.

#### Flash in the pan or long term threat? MPB in novel pine habitats

Stan Pokorny, University of British Columbia No abstract provided.

#### Manipulating Vector Competence in the Yellow Fever Mosquito, Aedes aegytpi

Lea Sanchez Milde, Heather Coatsworth, and Carl Lowenberger, Simon Fraser University

Aedes aegypti is the principal vector of dengue viruses. We are using CRISPR-Cas9 technology to knock out specific mosquito genes to generate dengue-refractory mosquitoes. We then will evaluate fitness and vector competence of the knockout lines to determine their suitability for use in dengue reduction programs.

#### Effect of nutrition status on the lifespan and reproductive output of the click beetle Agriotes obscurus

Kari Zurowski<sup>1</sup>, Jenny Cory<sup>1</sup>, Jessi Ly<sup>1</sup>, Danielle White<sup>2</sup>, Todd Kabaluk<sup>3</sup>, Alida Janmaat<sup>2</sup>, <sup>1</sup>Simon Fraser University, <sup>2</sup>University of the Fraser Valley, <sup>3</sup>Agriculture and Agri-Food Canada

Adult A. obscurus were paired and provided with an apple slice (fed) or no apple (starved) to determine the effect of nutrition on reproduction. Egg numbers and oviposition were recorded. Starved females laid fewer eggs for a shorter period than fed females, suggesting nutrition is important for A. obscurus reproduction.

## Trade-offs between reproduction and disease resistance in the click beetle Agriotes obscurus

Kari Zurowski<sup>1</sup>, Jenny Cory<sup>1</sup>, Jessi Ly<sup>1</sup>, Danielle White<sup>2</sup>, Todd Kabaluk<sup>3</sup>, Alida Janmaat<sup>2</sup>, <sup>1</sup>Simon Fraser University, <sup>2</sup>University of the Fraser Valley, <sup>3</sup>Agriculture and Agri-Food Canada

Adult *A. obscurus* were challenged with a high concentration, a low concentration, or a control of *M. brunneum* and their reproduction was monitored. Egg numbers and oviposition were recorded. Females challenged with the pathogen laid fewer eggs for a shorter amount of time than unchallenged insects, suggesting lifespan restricted fecundity.

## Effect of duration and location of pheromone trap placement in field margins on population estimates of two click beetle species

Wim Van Herk, Agriculture & Agri-Food Canada

Pheromone traps can be used to approximate the population size of pest click beetle species in areas where their larvae (wireworms) cause extensive damage to field crops (e.g. in PEI). If trap catches are used for making decisions in an IPM program for wireworms, it is important to know under what conditions trap catches are representative of the beetle populations present. The main pest click beetle species in Canada disperse primarily by walking, and hence it is likely that keeping a pheromone trap in a permanent location (e.g. in field margins) causes the population immediately around it to be depleted. Hence depending on how long they are maintained in a fixed location in the field, traps may underestimate actual populations. In this talk we demonstrate that this occurs, is affected by weather, and that it varies with species.

#### A trait-based approach to predicting spread rates of invasive forest insects

Brian Van Hezewijk & Lara Van Akker, Natural Resources Canada, Pacific Forestry Centre

Being able to predict how fast an invasive species will spread is crucial information for the management of novel alien species. Based on an historical database that documents the invasion of Canada's forests by 329 species of arthropods, we developed a statistical model that incorporates biological traits as well as geographic variables to predict the asymptotic rate of spread of new invaders.

# Symposium Abstracts: Biological Control — A Safe Approach to Pest Management

# Entomological Society of British Columbia Annual General Meeting Kwantlen Polytechnic University, Langley, BC, October 14, 2017

#### Biological control in cannabis

Amanda Brown, Biobest Canada Ltd.

I presented an overview of the beneficial insects that are currently used in indoor *Cannabis* production in Canada and the USA. Biological control programs are widely used because of the limited number of pesticides that can be used for insect pest control in order to comply with Health Canada and state regulations. The main pests in this crop are thrips, spider mites, fungus gnats — and occasionally russet mites, broad mites, and root aphids. Many biocontrol agents — including predatory mites, soil predators, minute pirate bugs, nematodes and parasitoids — are used for successful control of these pests.

#### Insect pathogens as biological control agents

Jenny S. Cory, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia

Insect pathogens have been studied for over 100 years, initially because of their negative effects on insects of economic interest, such as silkworms, and more recently as agents for pest management. The most studied insect pathogens are the entomopathogenic fungi, bacteria belonging to the Bacillus species, baculoviruses and entomopathogenic nematodes. All of these pathogen groups occur naturally in insect populations and many can cause wide-scale epizootics in their hosts. For example, when the locally common western tent caterpillar, a cyclic species, periodically reaches very high numbers, they will invariably die of a baculovirus infection. The insect pathogens used for pest management have narrow host ranges; all are restricted to insects, most only infect a few species and many are host specific. The most commercially successful microbial insecticide is the bacterium Bacillus thuringiensis, but representatives of all the major pathogen groups are available commercially. Insect pathogens tend to be used like chemical insecticides in that they are applied to high density pest populations. They are commonly used in forestry and for greenhouse crops, and they have also been widely used in crops such as maize, soybean and cotton and have been developed for fruit crops such as apples and pears. Their main advantages are that they are not toxic and have a narrow host range, and thus do not cause environmental damage or have health effects on humans. They could potentially be used more widely with the development of more novel pest management strategies which use their ecology, for example, their capacity to be dispersed and re-cycle naturally in pest populations.

#### Invasive insects: Is biological control an option?

Tracy Hueppelsheuser, British Columbia Ministry of Agriculture

Invasive species impact North American ecosystems both managed and unmanaged in significant ways. The Centre for Invasive Species Research in California states that invasive species cost California \$3 billion/year, every 60 days a new invasive enters California, and 6 new invasive species establish each year in California. Many new species to BC come up from initial introductions in California. Additionally, An Invasive Alien Species Strategy for Canada (2004) states that invasive alien species are the second most significant threat to biodiversity, after habitat loss. Canada has a long history of non-native introductions. The earliest record is Codling Moth (*Cydia pomonella*) in Ontario in 1635. The Canadian Food Inspection Agency states that invasive introductions

are on the increase due to increasing volume of trade, access to international markets, tourism and other travel, and decreasing transportation time.

The population of a new species in a new niche or location follows a sigmoidal curve, with the first few years being sub-detection, followed by some years of rapid increase in numbers which is usually when the new species is detected, and finally after some years will reach the carrying capacity of the new environment. Biological control agents, either naturally occurring or introduced, can play a role in decreasing the carrying capacity of the new environment, and ideally keeping the new species below acceptable levels where it doesn't cause significant damage. New or invader species may be more or less prone to control with biocontrol agents in the new environment, but most will fall somewhere in the middle.

Some examples of major new invaders to North America which are having an impact on agriculture crop production as well as urban landscapes are Spotted Wing Drosophila (Drosophila suzukii), Brown Marmorated Stink Bug (Halyomorpha halys), other stink bugs and leafhoppers. In all these cases, biocontrol probably has the best fit as part as a multi-faceted systems approach to overall crop and ecosystem management. example, many ideas are being explored or currently contributing to D. suzukii management, including more precise insecticide use, cultural and mechanical methods, exploitation of insect behaviour, in addition to exploring impacts and utility of entomopathogens, predators, and parasites on this new pest to berries and stone fruit. In the case of D. suzukii and H. halys, biological control with native parasitoids is low, generally less than 2%. Reasons include the fact that parasitoids aer not used to searching for hosts in the new niche that D. suzukii utilizes (ripe fruit vs decaying fruit), and that D. suzukii is especially good at encapsulating parasitoids, preventing them from developing. In the case of *H. halys*, native parasitoids do recognise the egg masses as suitable and oviposit in them, but unfortunately, the progeny cannot develop. Though it is a long and arduous process, biologically and from a regulatory perspective, there are significant efforts by researchers in the USA and Canada to screen and test suitable specialist parasitoids from the countries of pest origin in Asia. Suitable candidates will confer much higher levels of parasitism, and enable classical or innundative release as a practical component of pest management.

## Understanding insect oviposition behaviour and its influence on purple loosestrife biocontrol success

Alida F. Janmaat, Biology Department, University of the Fraser Valley

Patterns of oviposition can be used to elucidate the role of biotic and abiotic factors in the oviposition decisions made by insect biocontrol agents. Findings from a longterm study on the oviposition patterns of *Galerucella calamariensis*, leaf-feeding beetles released to control purple loosestrife, were presented. These findings coupled with laboratory experiments suggest that female oviposition choices made on the level of an individual plant may provide explanations for variation in biocontrol success observed across sites. Furthermore, the relationships observed suggest that cannibalism may play an under-appreciated role in the persistence of biocontrol insects in the field.

#### What is biological control and why do we need it?

Judith H Myers, Biodiversity Research Centre, University of British Columbia, Vancouver, BC

Biological control broadly defined is any non-chemical control. Generally biological control involves the use of natural enemies to control pest species. Five types of biological control were discussed at the symposium: 1. natural control, 2. augmentative control based on the release of natural enemies that have been collected or reared for this purpose, 3. conservation control in which habitat is preserved to maintain populations of natural enemies in the vicinity of agricultural fields, 4. microbial control using fungus, bacteria, virus or nematodes, and 5. classical biological control of exotic insects and

weeds through the release of natural enemies from the area where the pest is native. Classical biological programs are expensive and are taken on when there is evidence that the problem is of sufficient economic or ecological cost to warrant the expense and there is wide support for the program. Although host testing precedes releases of biological control agents, concerns about nontarget impacts have resulted in fewer programs being initiated in the last 20 years. Classical biological control programs have been successful in British Columbia for the weeds hounds tongue, tansy ragwort, diffuse knapweed, St. John'swort, and Dalmatian toadflax and for the winter moth. Currently programs for the release of biological control agents on knotweed and spotted wing drosophila are underway. It is important that classical biological control remains in the toolbox for dealing with exotic pests in the future.

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